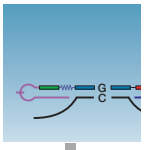
Applied Biosystems

Getting Started Guide

Chapter 1

Chapter 2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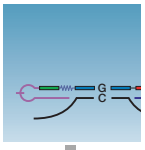
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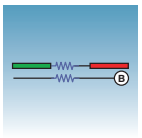
Chapter 7

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NOTICE TO PURCHASER

PLEASE REFER TO THE SNPlex™ GENOTYPING SYSTEM 48-PLEX USER GUIDE FOR LIMITED LICENSE OR DISCLAIMER INFORMATION

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Preface

How to Use This Guide

Purpose of This Guide

This guide provides a representative workflow using the SNPlex™ Genotyping System 48-plex with the Biomek FX system. It provides information to assist you in automating the Oligonucleotide ligation assay (OLA) using the Biomek FX instrument and is intended to be used with the *SNPlex™ Genotyping System 48-plex User Guide* (PN 4360856).

Audience

This guide is written for principal investigators and laboratory staff who intend to use the SNPlex™ Genotyping System 48-plex with robotics.

Assumptions

This guide assumes that you have read the *SNPlex™ Genotyping System 48-plex User Guide* and the *SNPlex™ Genotyping System 48-plex General Automation Getting Started Guide* (PN 4363143) and that you have a working knowledge of the assays and methods used for the SNPlex Genotyping System 48-plex.

Text Conventions

- **Bold** indicates user action. For example:

  Type **0**, then press **Enter** for each of the remaining fields.
- *Italic* text indicates new or important words and is also used for emphasis. For example:

  Before analyzing, *always* prepare fresh matrix.
- A right arrow bracket (>) separates successive commands you select from a drop-down or shortcut menu. For example:

  Select **File > Open > Spot Set**.

User Attention Words

Two user attention words appear in Applied Biosystems user documentation. Each word implies a particular level of observation or action as described below:

Note: Provides information that may be of interest or help but is not critical to the use of the product.

IMPORTANT! Provides information that is necessary for proper instrument operation, accurate chemistry kit use, or safe use of a chemical.

Examples of the user attention words appear below:

Note: The size of the column affects the run time.

IMPORTANT! To verify your client connection to the database, you need a valid Oracle user ID and password.

How to Obtain More Information

For more information about using the SNPlex Genotyping System 48-plex, refer to the:

- *SNPlex™ Genotyping System 48-plex User Guide* (PN 4360856)
- *SNPlex™ Genotyping System 48-plex Quick Reference Card* (PN 4360855)
- *SNPlex™ Genotyping System 48-plex Assay Design and Ordering Guide* (PN 4357460)
- *SNPlex™ Genotyping System 48-plex General Automation Getting Started Guide* (PN 4363143)
- *SNPlex™ Genotyping System 48-plex Automating PCR Using the Tomtec Quadra 3 Getting Started Guide (PN 4358100)*

Send Us Your Comments

Applied Biosystems welcomes your comments and suggestions for improving its user documents. You can e-mail your comments to:

techpubs@appliedbiosystems.com

How to Obtain Support

To contact Applied Biosystems Technical Support from North America by telephone, call **1.800.899.5858**.

For the latest services and support information for all locations, go to **http://www.appliedbiosystems.com**, then click the link for **Support**.

At the Support page, you can:

- Search through frequently asked questions (FAQs)
- Submit a question directly to Technical Support
- Order Applied Biosystems user documents, MSDSs, certificates of analysis, and other related documents
- Download PDF documents
- Obtain information about customer training
- Download software updates and patches

In addition, the Support page provides access to worldwide telephone and fax numbers to contact Applied Biosystems Technical Support and Sales facilities.

Safety and EMC Compliance Information

Safety Conventions Used in This Document

Safety Alert Words

Four safety alert words appear in Applied Biosystems user documentation at points in the document where you need to be aware of relevant hazards. Each alert word–**IMPORTANT, CAUTION, WARNING, DANGER**–implies a particular level of observation or action, as defined below:

Definitions

IMPORTANT! – Indicates information that is necessary for proper instrument operation, accurate chemistry kit use, or safe use of a chemical.

CAUTION – Indicates a potentially hazardous situation that, if not avoided, may result in minor or moderate injury. It may also be used to alert against unsafe practices.

WARNING – Indicates a potentially hazardous situation that, if not avoided, could result in death or serious injury.

Chemical Hazard Warning

WARNING **CHEMICAL HAZARD.** Before handling any chemicals, refer to the Material Safety Data Sheet (MSDS) provided by the manufacturer, and observe all relevant precautions.

About MSDSs

Chemical manufacturers supply current Material Safety Data Sheets (MSDSs) with shipments of hazardous chemicals to *new* customers. They also provide MSDSs with the first shipment of a hazardous chemical to a customer after an MSDS has been updated. MSDSs provide the safety information you need to store, handle, transport, and dispose of the chemicals safely.

Each time you receive a new MSDS packaged with a hazardous chemical, be sure to replace the appropriate MSDS in your files.

Obtaining MSDSs

You can obtain from Applied Biosystems the MSDS for any chemical supplied by Applied Biosystems. This service is free and available 24 hours a day.

To obtain MSDSs:

1. Go to **https://docs.appliedbiosystems.com/msdssearch.html**
2. In the Search field, type in the chemical name, part number, or other information that appears in the MSDS of interest. Select the language of your choice, then click **Search**.
3. Find the document of interest, right-click the document title, then select any of the following:
   - **Open** – To view the document
   - **Print Target** – To print the document
   - **Save Target As** – To download a PDF version of the document to a destination that you choose

Chemical Safety Guidelines

To minimize the hazards of chemicals:

- Read and understand the Material Safety Data Sheets (MSDS) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. (See "About MSDSs" on page vii.)
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing). For additional safety guidelines, consult the MSDS.
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood). For additional safety guidelines, consult the MSDS.
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures as recommended on the MSDS.
- Comply with all local, state/provincial, or national laws and regulations related to chemical storage, handling, and disposal.

Chemical Waste Safety

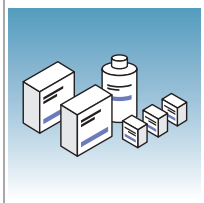
Chemical Waste Hazard

CAUTION **HAZARDOUS WASTE.** Refer to Material Safety Data Sheets (MSDSs) and local regulations for handling and disposal.

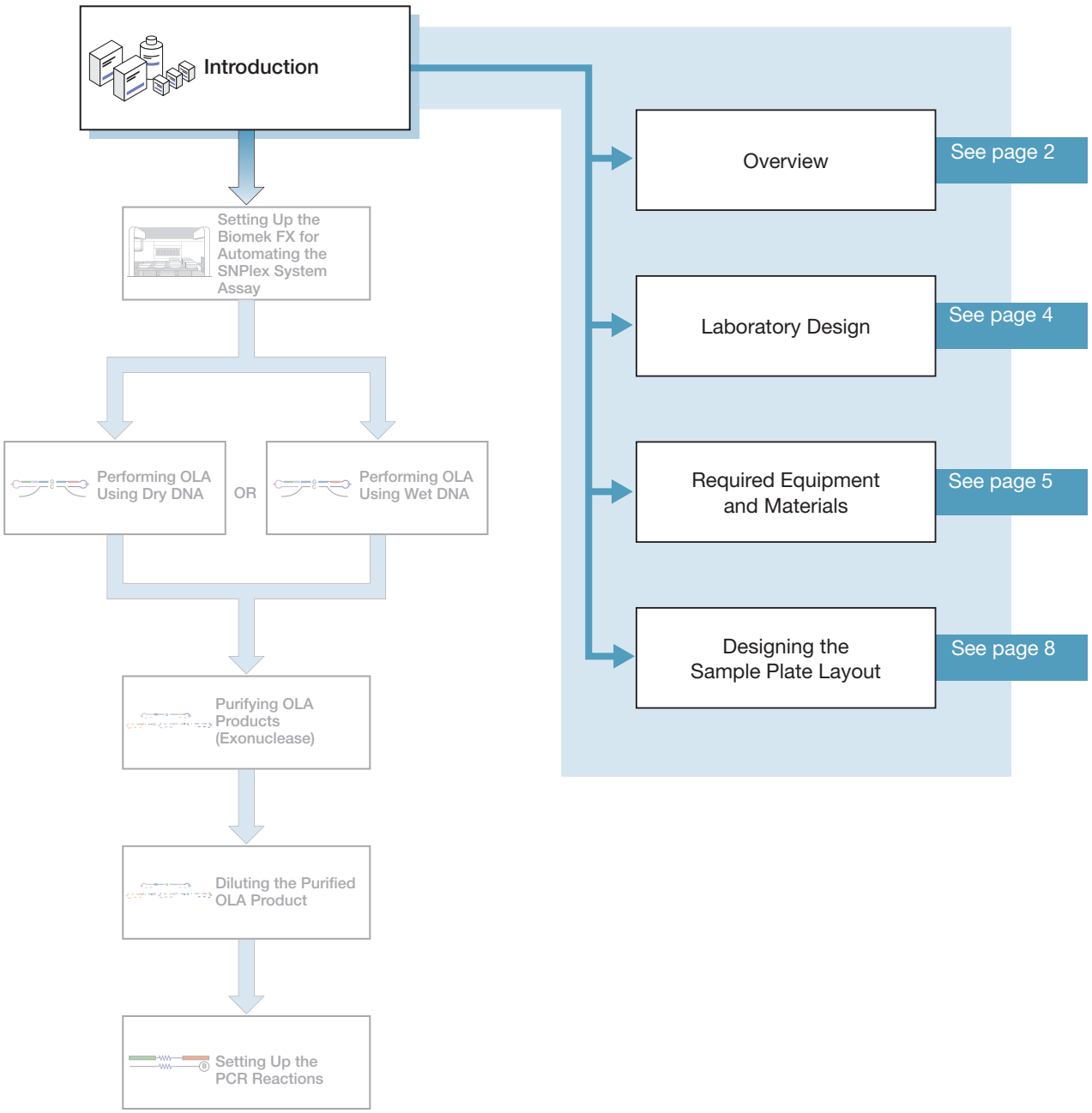
Chemical Waste Safety Guidelines

To minimize the hazards of chemical waste:

- Read and understand the Material Safety Data Sheets (MSDSs) provided by the manufacturers of the chemicals in the waste container before you store, handle, or dispose of chemical waste.
- Provide primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing). For additional safety guidelines, consult the MSDS.
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood). For additional safety guidelines, consult the MSDS.
- Handle chemical wastes in a fume hood.
- After emptying the waste container, seal it with the cap provided.
- Dispose of the contents of the waste tray and waste bottle in accordance with good laboratory practices and local, state/provincial, or national environmental and health regulations.



Introduction



Notes

Overview

The SNPlex™ System assay consists of several protocols which involve manipulating small volumes between 96- and 384-well plates and reservoirs. This guide contains the protocols required to complete the oligonucleotide ligation assay (OLA) portion of SNPlex System assays using the Biomek FX System.

About This Guide

The *SNPlex™ Genotyping System 48-plex Automating OLA Using the Biomek FX Getting Started Guide* provides instructions for automating the OLA protocols using:

- The Biomek FX System. For information about automating the OLA procedures using other instruments, refer to the appropriate guide. (See "Documentation" on page 7.)
- The 384-well protocol. For information about the 96-well protocol, refer to the *SNPlex™ Genotyping System 48-plex User Guide* (PN 4360856) for instructions.
- The Applied Biosystems 3730*xl* DNA Analyzer to collect data.
- GeneMapper® software v3.7 to analyze data.

Assumptions

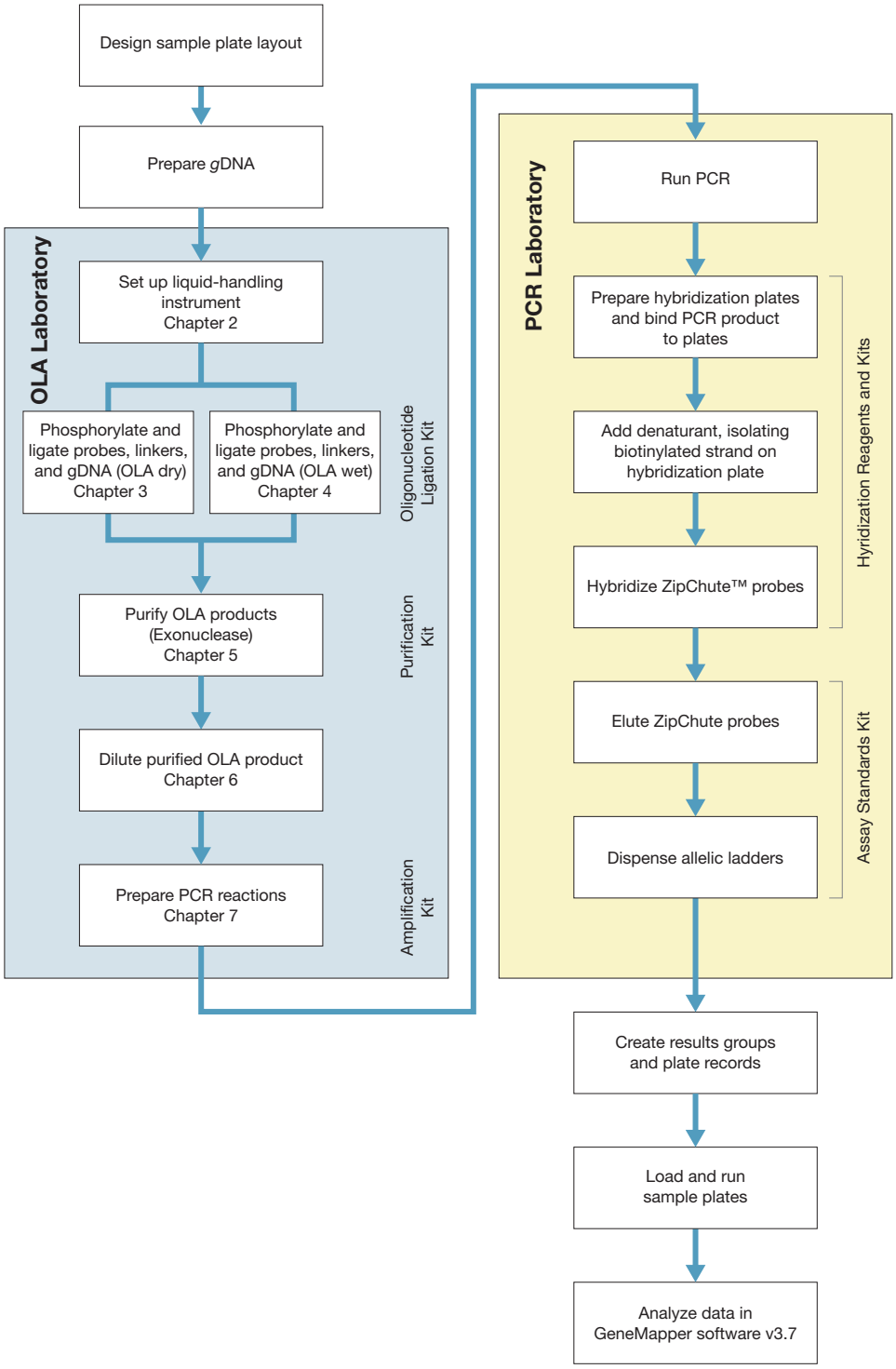
This guide assumes that you have read the *SNPlex™ Genotyping System 48-plex User Guide* and the *SNPlex™ Genotyping System 48-plex General Automation Getting Started Guide* (PN 4363143) and that you have a working knowledge of the assays and methods used for the SNPlex Genotyping System 48-plex.

This guide also assumes that you have a working knowledge of the operation of the Biomek FX, including set up, framing, and building of the deck.

Notes

SNPlex System Automation Workflow

The following diagram illustrates the SNPlex System workflow.

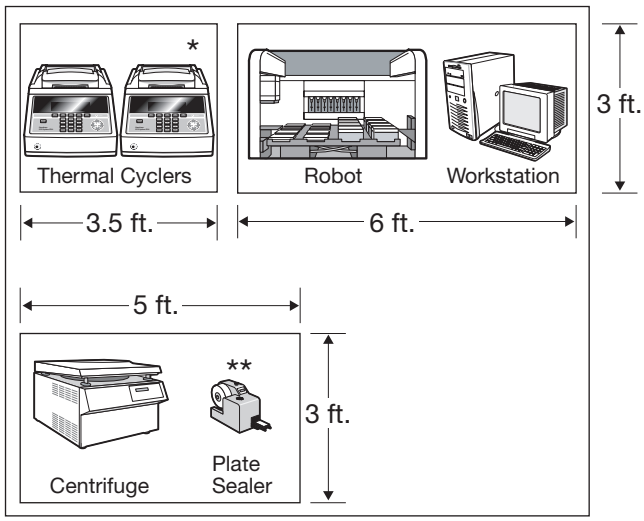

Design sample plate layout
Prepare gDNA
OLA Laboratory
Set up liquid-handling instrument Chapter 2
Phosphorylate and ligate probes, linkers, and gDNA (OLA dry) Chapter 3
Phosphorylate and ligate probes, linkers, and gDNA (OLA wet) Chapter 4
Oligonucleotide Ligation Kit
Purify OLA products (Exonuclease) Chapter 5
Purification Kit
Dilute purified OLA product Chapter 6
Prepare PCR reactions Chapter 7
Amplification Kit
PCR Laboratory
Run PCR
Prepare hybridization plates and bind PCR product to plates
Add denaturant, isolating biotinylated strand on hybridization plate
Hybridize ZipChute™ probes
Hyridization Reagents and Kits
Elute ZipChute probes
Dispense allelic ladders
Assay Standards Kit
Create results groups and plate records
Load and run sample plates
Analyze data in GeneMapper software v3.7

Notes

Laboratory Design

The protocols contained in this guide should be performed in an amplicon-free OLA lab, such as that shown in the following figure.

OLA Lab Layout



* This is a vertical shelf unit, depending on the number of shelves, can house 2 thermal cyclers per shelf.

** Optional

For additional information about laboratory design, refer to the *SNPlex™ Genotyping System 48-plex General Automation Getting Started Guide*.

Plate Sealing

A plate sealer is recommended but not required.

Applied Biosystems has found that certain plate covers negatively affect the performance of the SNPlex System assay. If you do not use a plate sealer, you may use one of the recommended plate covers listed in Table 1-3 on page 1-9 of the *SNPlex™ Genotyping System 48-plex User Guide*. If you use covers other than the recommended plate covers, test them using the SNPlex™ System Control Set (see Appendix A of the *SNPlex™ Genotyping System 48-plex User Guide*).

Notes

Required Equipment and Materials

Equipment

Item	Vendor	Part Number
GeneAmp® PCR System 9700 Dual 384-Well Sample Block Module	Contact your Applied Biosystems representative for information.	
Biomek FX Single Arm System with:	Contact your Beckman Coulter Representative	
• 96-channel 20 µL disposable tip pipetting head • Disposable tip loader ALP • 2 Standard single position ALPs • 16-position (4X4) high-density ALP	Beckman Coulter	719367 719356 719357 719360
Centrifuge (equipped to accommodate reaction plates)	Major Laboratory Supplier (MLS)	–
Sealer for microtiter plates. Recommend: • ALPS-300 Heat Sealer • Air compressor with clean air package • Easy Peel Foil Sealing Film for ALPS-300	ABGene	 AB-0950 CMP-950 AB-3739

Consumables and Small Equipment

Item		Vendor	Part Number
15-mL centrifuge tubes		MLS	–
384-well plate adapters, eight		Acme Automation	CR7019
50-µL Barrier, 96-channel disposable tip boxes		Axygen	FXF-50-L-R-S
Multichannel pipettor, 250-µL		MLS	–
Pipettors		MLS	–
Pipetting resevoirs, 25-mL		MLS	–
Pipetting resevoirs, 100-mL		MLS	–
Reaction Plates	ABI PRISM® 384-Well Clear Optical Reaction Plate, with Barcode, 50 plates	Applied Biosystems	4309849
	ABI PRISM® 384-Well Optical Reaction Plate with Barcode, 500 plates	Applied Biosystems	4326270

Notes

Item (*continued*)		Vendor	Part Number
Reaction Plate Covers†	MicroAmp® Full 96-Well Plate Cover	Applied Biosystems	N8010550
	ABI PRISM™ Optical Cover Compression Pad	Applied Biosystems	4312639
• Heat seals and sealers	Easy-Peel individual sheets	ABGene	AB-0745
	Easy-Peel 610 meter roll	ABGene	AB-3739
	UNISEAL AL	Whatman	7704-0002
	Thermosealer	ABGene	AVB-0384
	Plate Sealer, ALPS 300™	ABGene	AB-0950
• Adhesive seals	384 Well Microplate Aluminum Sealing Tape	Corning	6569
	Adhesive PCR foil seal	ABGene	AB-0626
	SILVERseal™	Greiner	676 090
Reagent reservoir		Axygen	RES-SW96-HP
Skirted 96-well PCR plates		Axygen	PCR-96-FS-C
Vortex		MLS	–

† IMPORTANT! Applied Biosystems has found that certain plate covers negatively affect the performance of the SNPlex System assay. If you use covers other than the recommended plate covers, test them using the SNPlex™ System Control Set (see Appendix A of the SNPlex™ Genotyping System 48-plex User Guide).

Kits and Reagents

Item	Vendor	Part Number
SNPlex System Kits Required for This Protocol		
SNPlex™ System Oligonucleotide Ligation Kit • SNPlex™ OLA Master Mix • SNPlex™ Universal Linkers, 48-plex • SNPlex™ dATP, 100×	Applied Biosystems	4357460
SNPlex™ System Ligation Probes	Applied Biosystems	4346978
SNPlex™ System Purification Kit • SNPlex™ Exonuclease Buffer, 10× • SNPLex™ Lamda Exonuclease • SNPlex™ Exonuclease I	Applied Biosystems	4349357
SNPlex™ System Amplification Kit • SNPlex™ Amplification Master Mix, 2× • SNPlex™ Amplification Primers, 20×	Applied Biosystems	4349358
Other Reagents		
Nuclease-free water	Promega	P119C

Notes

Item	Vendor	Part Number
Sterile 1X TE buffer (10 mM Tris-base, pH 8.0, and 1 mM Na_2EDTA)	Fluka	93283

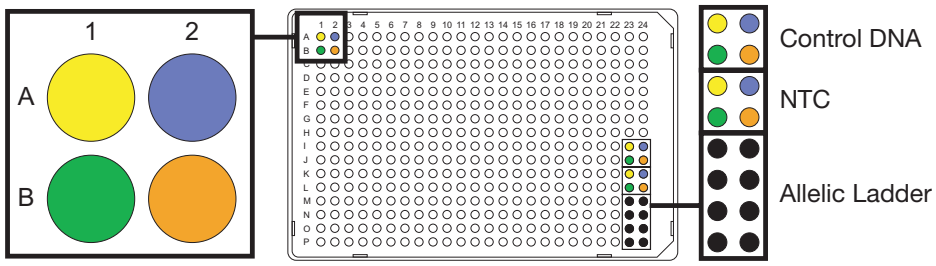
Documentation

Document Name	Part Number
SNPlex™ Genotyping System 48-plex Assay Ordering Guide	4357460
SNPlex™ Genotyping System 48-plex User Guide	4360856
SNPlex™ Genotyping System 48-plex Quick Reference Card	4360855
SNPlex™ Genotyping System 48-plex General Automation Getting Started Guide	4363143
Biomek FX User's Manual	Beckman Coulter 719452
SNPlex™ Genotyping System 48-plex Automating PCR Using the Tomtec Quadra 3 Getting Started Guide	4358100

Notes

Designing the Sample Plate Layout

Each 384-well plate is divided into four quadrants, each with 96 wells. The convention used in this document is that the first of 96 wells in quadrant 1 is well A1, well B1 for quadrant 2, A2 for quadrant 3, and B2 for quadrant 4.



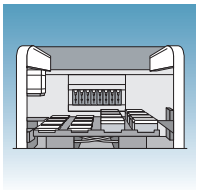
A1 = Quadrant 1 A2 = Quadrant 3
B1 = Quadrant 2 B2 = Quadrant 4

A number of plate layouts are possible, assuming that each batch consists of four 384-well plates [as illustrated in "Plate Layouts and Methods for the OLA Protocol (Dry gDNA)" on page 21 and "Plate Layouts and Methods for the OLA Protocol (Wet gDNA)" on page 37.]

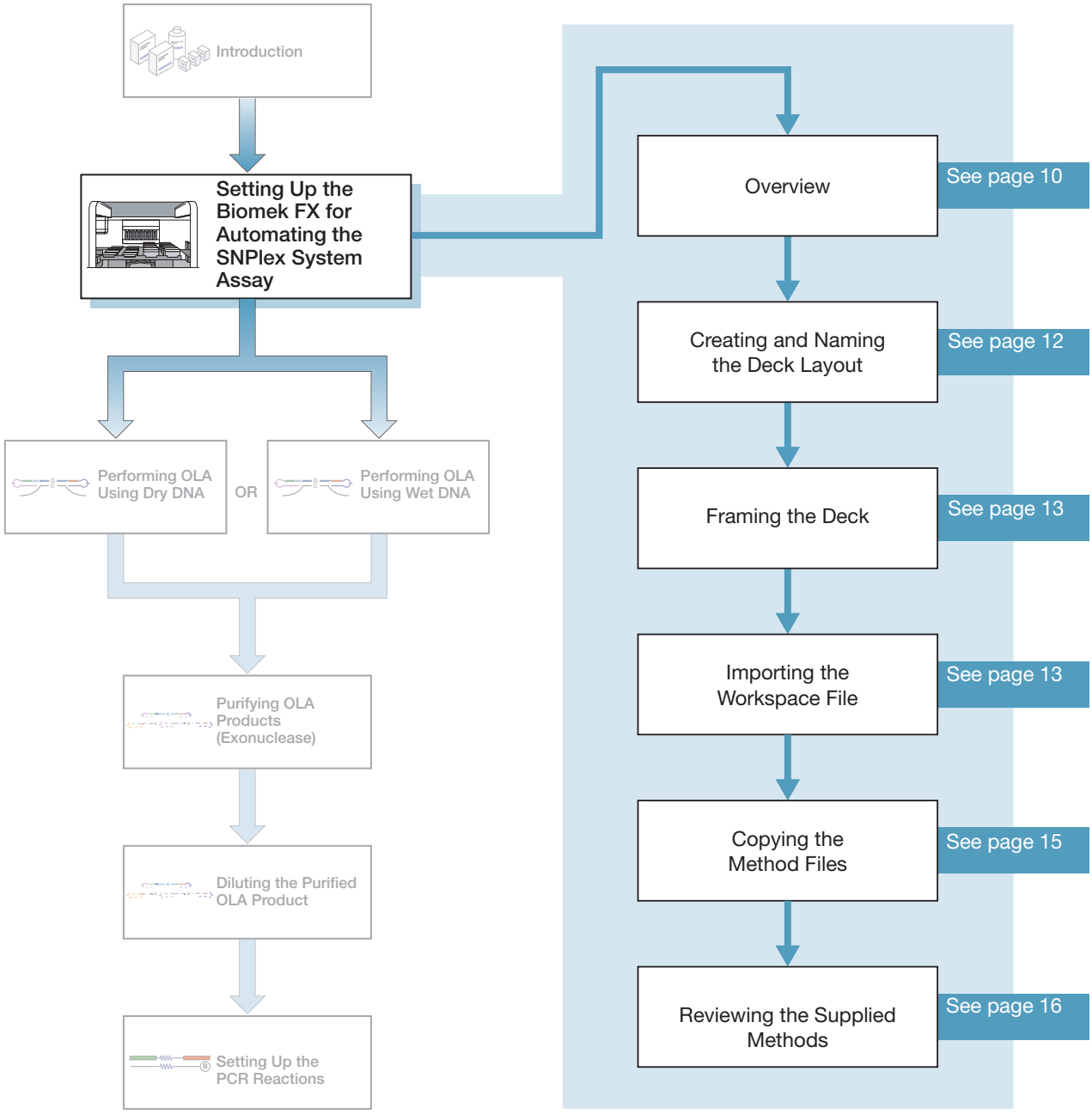
The layout of a sample plate must be coordinated with the structure and naming of Data Collection software run folders in order for GeneMapper® software to correctly analyze the data. Each run must include an allellic ladder, control DNA, and no template control (NTC).

For additional information about sample plate layout, refer to the *SNPlex™ Genotyping System 48-plex General Automation Getting Started Guide*. Note that different robotics manufacturers have differing conventions for the order and location of plate quadrants. Be cognizant of these differences when programming robotics.

Notes



Setting Up the Biomek FX for Automating the SNPlex System Assay



Notes

Overview

About Workspace and Method Files

Instructions for automating the SNPlex System assay are contained in two types of files:

- workspace
- method

Workspace Files

Workspace files contain labware and tip definitions, pipetting templates, techniques, and liquid types. They also contain settings needed by the Biomek FX software to run the method files.

The workspace file for the Biomek FX, AB_SNPLEX_Workspace.imp, contains the following settings:

Setting Type	Settings
Labware Definitions	snplex_OLA_ABI_384_in_ACME_Adapter
	snplex_PCR_ABI_384_in_ACME_Adapter
	snplex_VWR_96_PP_16posALP
	snplex_VWR_96_PP_1posALP
	snplex_Axygen_96_DiaBot_HP
	Axygen96_50uL_Barrier
Pipetting Templates	Default Template
	Low Volume
Techniques	snplex_AssayMix_3uL
	snplex_AssayMix_5uL
	snplex_DNA_2uL
	snplex_ExoMix_5uL
	snplex_ExoDil_5uL
	snplex_PCRMix_7.92uL
	snplex_OLARxn_2.08uL
Liquid Types	snplex_DNASample
	snplex_ExoMix
	snplex_AssayMix
	snplex_OLARxn
	snplex_PCRMix
Tips	AxyP50_Barrier

Notes

Method Files

Method files contain the instructions for a workflow activity on a robot. Method files are specific for a given robot and can be read only by the software of that robot. For example, the four methods provided for performing OLA using dry gDNA on the Biomek FX can be read only by the Biomek FX software.

Applied Biosystems provides a total of 11 methods for automating the pre-PCR process:

- Eight methods for the OLA setup, four for using dry gDNA and four for using wet DNA
- One method for the exonuclease purification step
- One method for the OLA dilution step
- One method for the PCR setup step

These methods are explained in succeeding chapters of this guide.

IMPORTANT! The sample plate layouts and methods provided in this document are designed for experiments using batches of four 384-well plates.

Summary

Setting up the Biomek FX for automating the OLA lab process involves five tasks:

- "Creating and Naming the Deck Layout" on page 12
- "Framing the Deck" on page 13
- "Importing the Workspace File" on page 13
- "Copying the Method Files" on page 15
- "Reviewing the Supplied Methods" on page 16

If you are using the Biomek FX instrument exclusively for the SNPlex System assay (or if you are using the SNPlex System workspace for all other applications), setting up the instrument is a one-time process. If you are using the instrument with other workspaces, you may need to repeat some steps in the setup process (such as importing the workspace file and reviewing the setup).

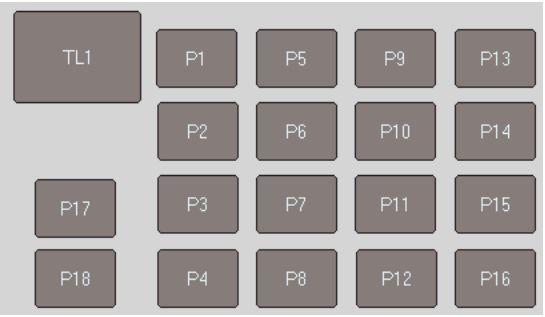
Notes

Creating and Naming the Deck Layout

Building the deck layout includes both physically building the deck on the instrument and modelling the physical deck in the Biomek FX software Deck Editor. In the Deck Editor, name the deck layout file "ABI_SNPLEX_NC."

IMPORTANT! Build the deck layout before the Installation and Operation Qualifications (IQ/OQ) are performed.

The following illustration shows the positions on an empty deck for the Biomek FX:



TL1 = Tip Loader
P1 to p16 = 16-position ALP (4 x 4)
P17 to P18 = 2 single position ALPs

In the figure above

- Positions P1 through P16 comprise a single 4✕4 hardware component (the 16-position ALP).
- Positions P17 and P18 are individual 1✕1 single-position ALPs.
- The tip loader ALP is at TL1.

Note: If you have a new Biomek FX instrument, a Beckman Coulter representative must perform IQ/OQ, using the physical deck layout and virtual deck layout that you just built.

Notes

Framing the Deck

Before you can use the Biomek FX instrument, you must calibrate the positions on the deck. Biomek FX documentation refers to this process as "framing the deck."

During this process, coordinates of each position on the deck are established, enabling the instrument to move objects to and from various locations on the deck precisely.

IMPORTANT! Framing the deck ensures the correct transfer of materials, which is essential to proper method operation. Do not use the Biomek FX instrument unless you have completed the deck-framing process.

Refer to the Biomek FX documentation for more information about this process.

Importing the Workspace File

Downloading the Zip Archive

Note: Because the zip archive contains both the workspace and method files, you need only download it once.

1. Go to http://www.appliedbiosystems.com.
2. Click **Support** at the top of the page.
3. On the Support page, click **Software Downloads**.
4. From the Select Product Software menu, select **SNPlex Genotyping System**.
5. From the Software Type menu, select **Main Page**.
6. Download the appropriate zip archive for your robot.

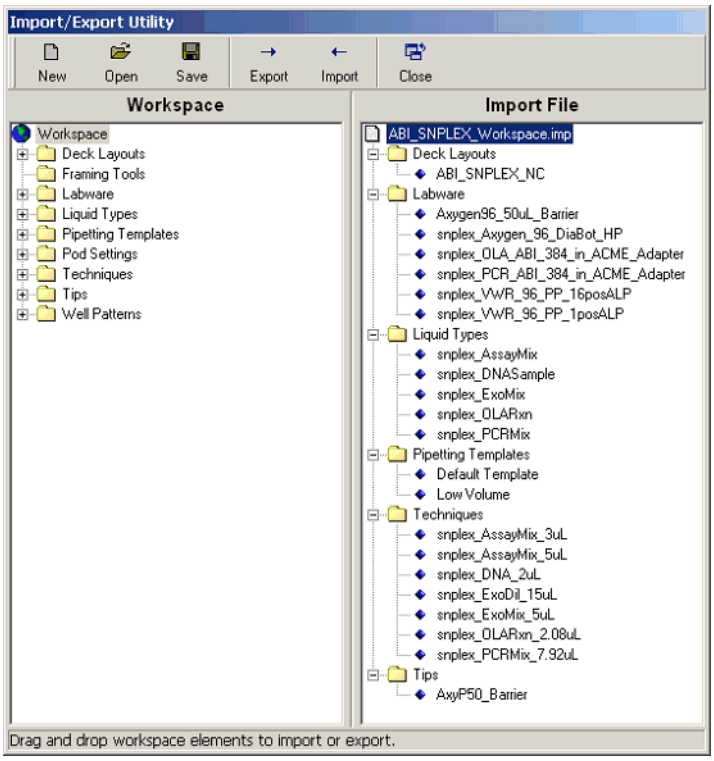
Importing the Workspace File

IMPORTANT! If you use the Biomek FX instrument for tasks other than automating the SNPlex System assay, back up your workspace before importing the SNPlex System workspace file. Workspace parameters for the SNPlex System assay may be different than those for other configurations and may overwrite those parameters. Refer to the Biomek FX software documentation for information on backing up a workspace.

1. Start the Biomek FX software.
2. Select **Tools > Import/Export Utility**.

Notes

3. In the Import/Export Utility window, click **Open**, then browse to the location to which you unzipped the workspace file, and select **ABI_SNPLEX_Workspace.imp**.



4. Click **Open** to display the SNPlex System workspace in the Import File pane.

5. Select **ABI_SNPLEX_Workspace.imp**, then click **Import**.

Notes

Copying the Method Files

1. Start the Biomek FX software.
2. Select **File > Open**, browse to the location to which you unzipped the method files, and select a method file.
3. Select **File >Save** to save the file to an appropriate location.
4. Repeat steps 3 to 5 until you have saved the following files:

1a_SNPLEXv5_OLA_PN_S4_D.bmt	1c_SNPLEXv5_OLA_P16_S1_W.bmt
1b_SNPLEXv5_OLA_P4_SN_D.bmt	1d_SNPLEXv5_OLA_P1_S16_W.bmt
1c_SNPLEXv5_OLA_P16_S1_D.bmt	2_SNPLEXv5_EXO.bmt
1d_SNPLEXv5_OLA_P1_S16_D.bmt	3_SNPLEXv5_EXO_DILUTION.bmt
1a_SNPLEXv5_OLA_PN_S4_W.bmt	4_SNPLEXv5_PCR.bmt
1b_SNPLEXv5_OLA_P4_SN_W.bmt	

Note: You can also use Windows Explorer to copy the files from the location to which you downloaded them to an appropriate location on the Biomek FX workstation

Notes

Reviewing the Supplied Methods

Verifying the Method Conditions

Because the proper operation of a method depends on the presence of specific conditions (such as the number of 384-well reaction plates), you should verify that the methods you copied have the specifications listed in the following table:

Method Number	Method File Name	Number of 384-well Reaction Plates	Number of 96-well Reagent Source Plates	Number of Reagent Reservoirs	Estimated Time (mm:ss)	Tip Boxes
1a_dry	1a_SNPLEXv5_OLA_PN_S4_D.bmt	4	4	—	4:16	4
1b_dry	1b_SNPLEXv5_OLA_P4_SN_D.bmt	4	4	—	4:19	4
1c_dry	1c_SNPLEXv5_OLA_P16_S1_D.bmt	4	16	—	11:25	16
1d_dry	1d_SNPLEXv5_OLA_P1_S16_D.bmt	4	1	—	2:24	1
1a_wet	1a_SNPLEXv5_OLA_PN_S4_W.bmt	4	8	—	15:31	20
1b_wet	1b_SNPLEXv5_OLA_P4_SN_W.bmt	4	8	—	15:32	20
1c_wet	1c_SNPLEXv5_OLA_P16_S1_W.bmt	4	17	—	13:48	17
1d_wet	1d_SNPLEXv5_OLA_P1_S16_W.bmt	4	17	—	13:49	17
2	2_SNPLEXv5_EXO.bmt	4†	1	—	12:39	16
3	3_SNPLEXv5_EXO_DILUTION.bmt	4†	—	1	13:08	16
4	4_SNPLEXv5_PCR.bmt	4† + 4	1	—	15:22	17

† Reaction plates are the output plates that are carried over from the previous method.

Note: Four methods are used to complete the OLA process. Select one of the eight OLA setup methods (that is, 1a_dry, 1a_wet, 1b_dry, 1b_wet, and so forth). The four OLA reaction plates are carried over into method 2 (Exo), then into method 3(Dilution), and then into method 4(PCR). In method 4, aliquots from the original four reaction plates are transferred into four new PCR reaction plates.

Notes

Verifying the Labware

Methods also require the use of specific labware in order to function properly. The following table lists the labware used to develop the Biomek FX methods.

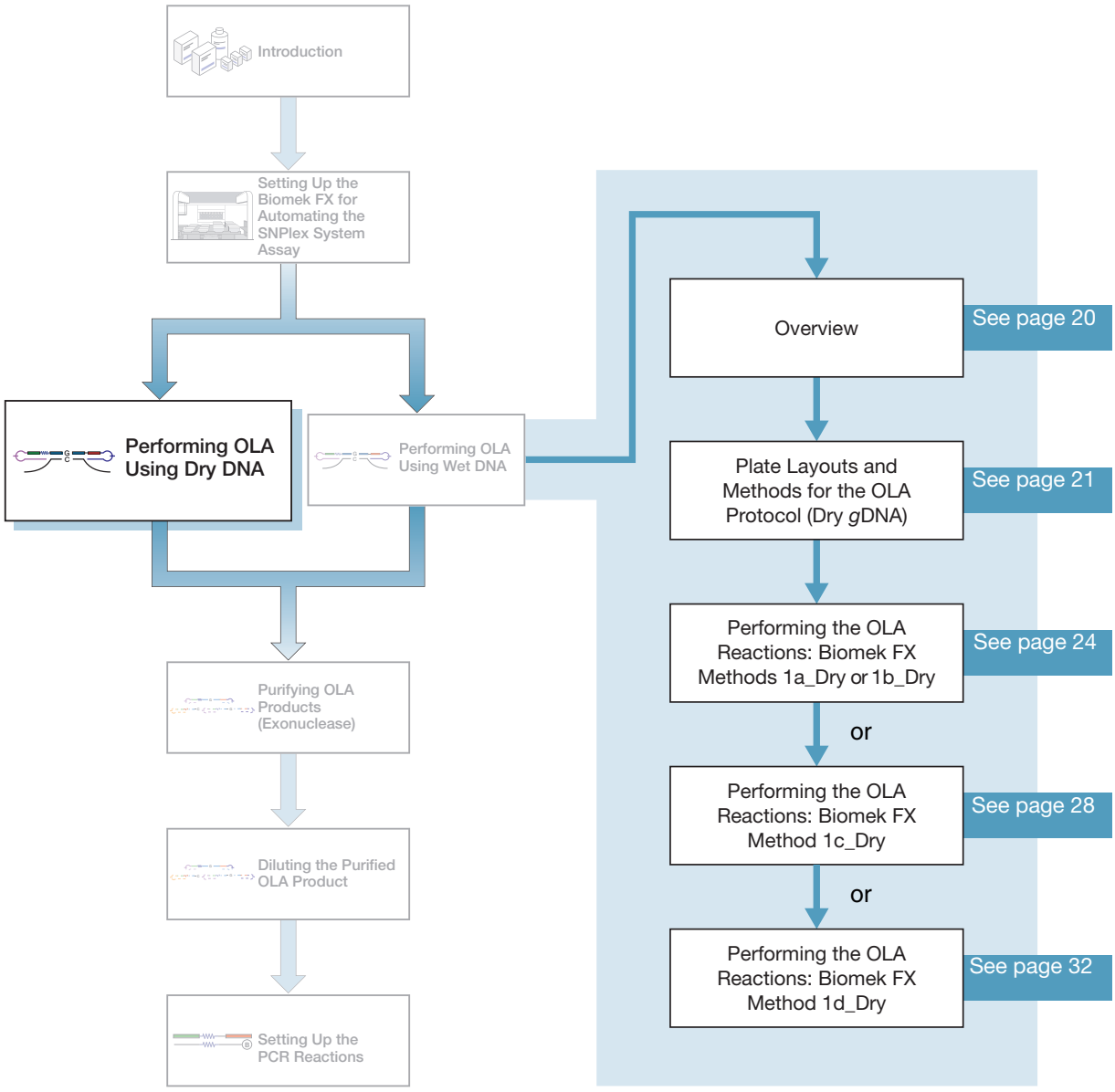
Description	Vendor	Title in Method
384-well reaction plate	Applied Biosystems 4309849	OLA_ABI_384_in_ACME_Adapter PCR_ABI_384_in_ACME_Adapter
Axygen 50-µL barrier tips	Axygen FXF-50-L-R-S	Axygen96_50uL_Barrier
Reagent reservoir	Axygen RES-SW96-HP	Axygen_96_DiaBot_HP
Skirted 96-well PCR plate	Axygen PCR-96-FS	VWR_96_PP_16posALP VWR_96_PP_1posALP

Once the setup is complete, you can start using the Biomek FX instrument for automating the OLA lab protocols of the SNPlex System assay, as described in the following chapters.

Notes

Notes

Performing OLA Using Dry gDNA


Introduction
Setting Up the Biomek FX for Automating the SNPlex System Assay
Performing OLA Using Dry DNA
Performing OLA Using Wet DNA
Purifying OLA Products (Exonuclease)
Diluting the Purified OLA Product
Setting Up the PCR Reactions
Overview
See page 20
Plate Layouts and Methods for the OLA Protocol (Dry gDNA)
See page 21
Performing the OLA Reactions: Biomek FX Methods 1a_Dry or 1b_Dry
See page 24
or
Performing the OLA Reactions: Biomek FX Method 1c_Dry
See page 28
or
Performing the OLA Reactions: Biomek FX Method 1d_Dry
See page 32

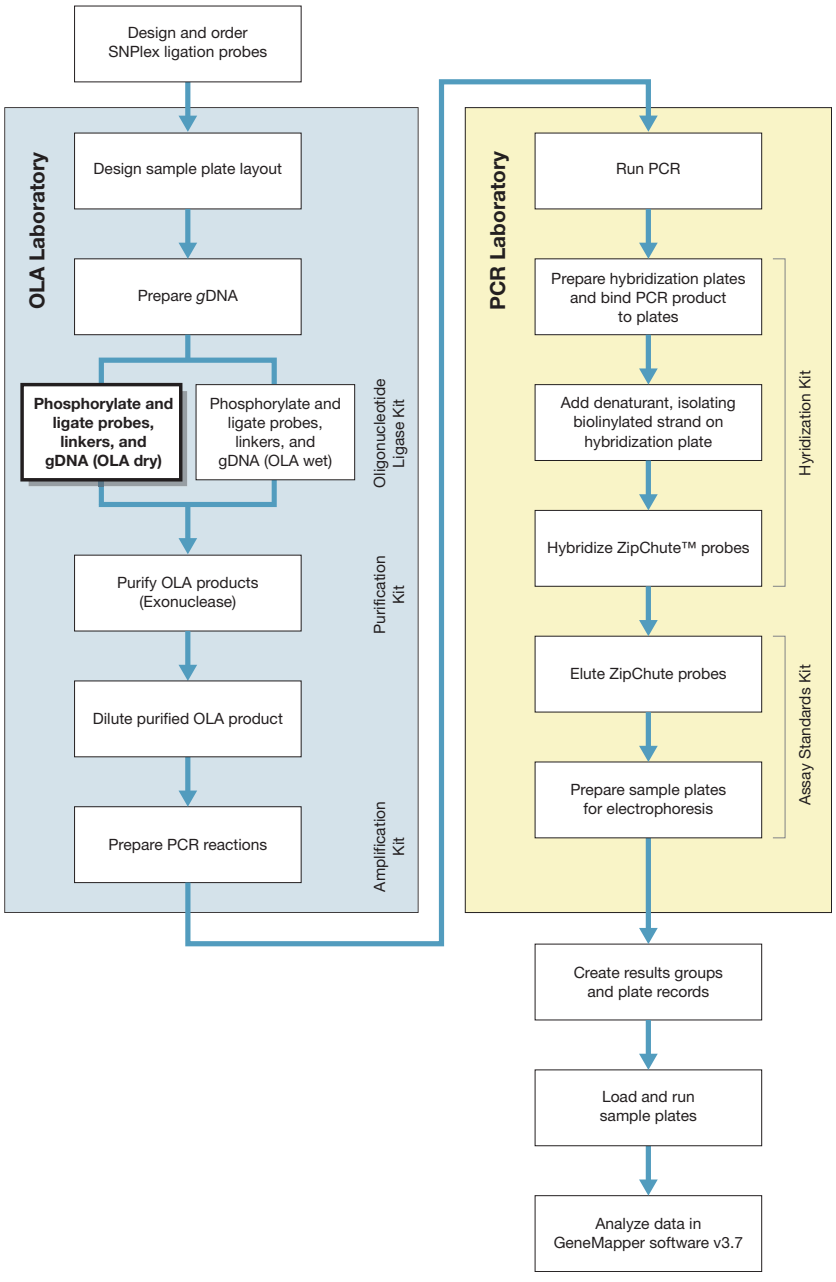
Notes

Overview

About This Chapter

This chapter provides information about automating the OLA protocol with the Biomek FX using disposable tips. The procedures in this chapter assume the use of a batch of four SNPlex™ OLA reaction plates, each containing 37 ng/well of dried, fragmented gDNA samples. If you are using wet gDNA, refer to Chapter 4, "Performing OLA Using Wet gDNA."

Where You Are In the SNPlex System Assay Workflow


Design and order SNPlex ligation probes
OLA Laboratory
Design sample plate layout
Prepare gDNA
Phosphorylate and ligate probes, linkers, and gDNA (OLA dry)
Phosphorylate and ligate probes, linkers, and gDNA (OLA wet)
Oligonucleotide Ligase Kit
Purify OLA products (Exonuclease)
Purification Kit
Dilute purified OLA product
Prepare PCR reactions
Amplification Kit
PCR Laboratory
Run PCR
Prepare hybridization plates and bind PCR product to plates
Add denaturant, isolating biolinylated strand on hybridization plate
Hybridize ZipChute™ probes
Hyridization Kit
Elute ZipChute probes
Prepare sample plates for electrophoresis
Assay Standards Kit
Create results groups and plate records
Load and run sample plates
Analyze data in GeneMapper software v3.7

Notes

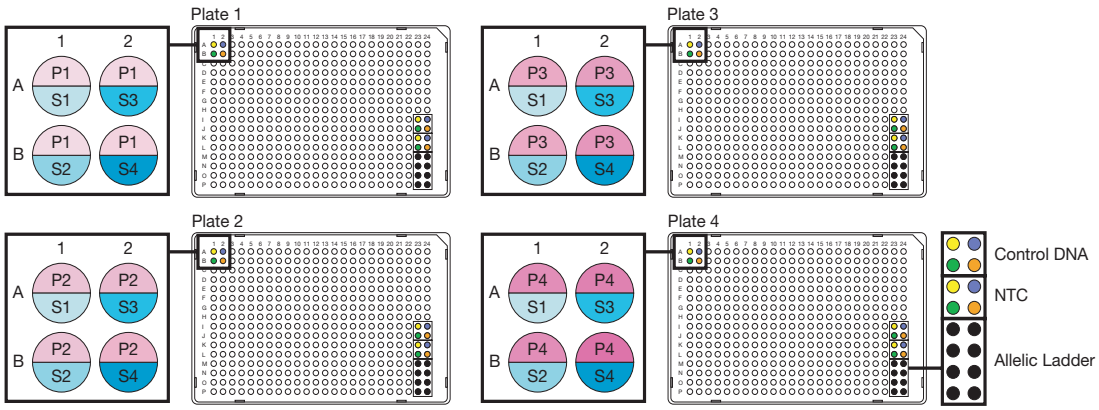
Plate Layouts and Methods for the OLA Protocol (Dry gDNA)

Reaction Plate Layouts

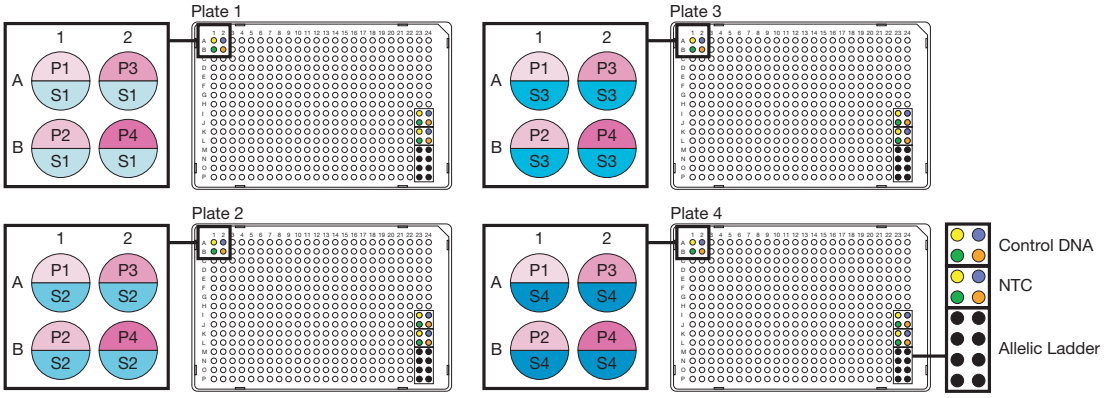
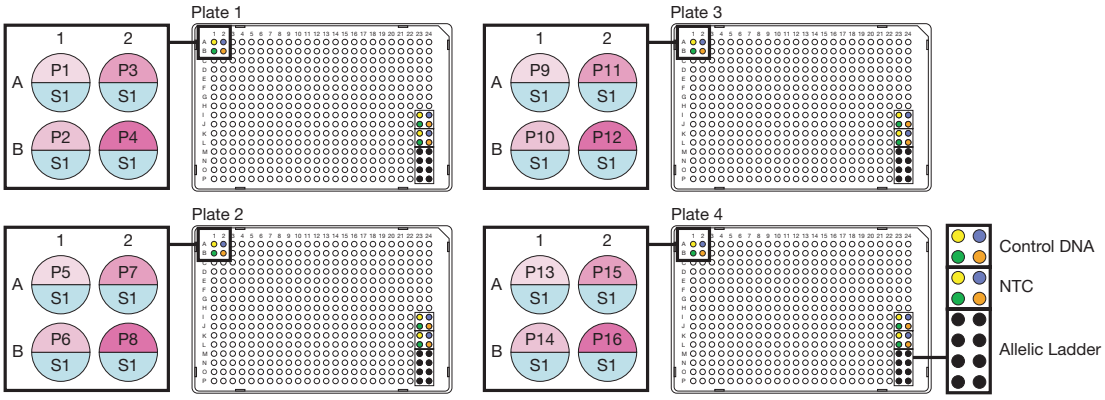
You can select from four Biomek FX methods depending on the number of samples and probe pools assayed in a single run. The following table gives the plate layouts and the associated file for each layout. The quadrant representation comes from the division of the 384-well reaction plates into four 96-well quadrants.

Although plate layouts are flexible, some layouts are more efficient with reagent usage than others. The layout that assays 1472 samples with a single probe pool is most efficient since reagent dead volume is limited to a single 96-well source container. The layout that assays 92 samples with 16 probe pools is the most inefficient since reagent dead volume is spread across sixteen 96-well source containers. For optimal use of SNPlex System reagent kits, consider plate layouts, batch sizes, and "dried vs wet" gDNA setup.

Method Number†	Method File Name‡	Number of Probe Pools	Total Number of Samples§	Reference
1a_dry	1a_SNPLEXv5_OLA_PN_S4_D.bmt	4	368	"Performing the OLA Reactions: Biomek FX Methods 1a_dry or 1b_dry" on page 24

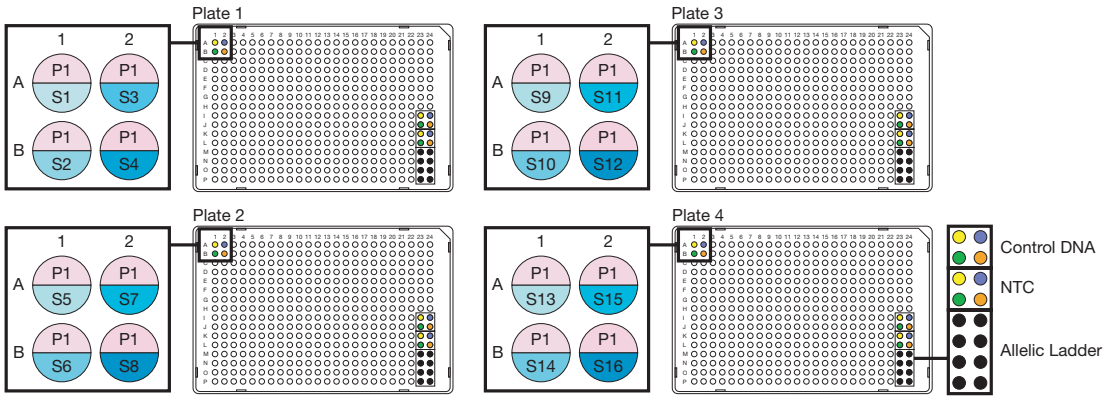


Notes

Method Number†	Method File Name‡	Number of Probe Pools	Total Number of Samples§	Reference
1b_dry	1b_SNPLEXv5_OLA_P4_SN_D.bmt	4	368	"Performing the OLA Reactions: Biomek FX Methods 1a_dry or 1b_dry" on page 24
				
1c_dry	1c_SNPLEXv5_OLA_P16_S1_D.bmt	16	92	"Performing the OLA Reactions: Biomek FX Method 1c_dry" on page 28
				

Notes

Method Number†	Method File Name‡	Number of Probe Pools	Total Number of Samples§	Reference
1d_dry	1d_SNPLEXv5_OLA_P1_S16_D.bmt	1	1472	"Performing the OLA Reactions: Biomek FX Method 1d_dry" on page 32



† Method numbering reflects methods provided for other SNPlex System assay protocols.
‡ P = Probe Pool; S = Unique collection of 92 gDNA samples (4 positions of a 96-well quadrant are reserved for 1 control DNA, 1 NTC, and 2 allelic ladder wells).
§ Total number of samples in a batch containing four 384-well plates.

Notes

Performing the OLA Reactions: Biomek FX Methods 1a_dry or 1b_dry

About These Methods

Methods 1a_dry and 1b_dry were developed for setups that have 368 gDNA samples and four probe pools.

These methods use a single identical deck layout. The difference between them is the pattern in which the instrument dispenses the reagents into the wells of the OLA reaction plates.

In Method 1a_dry:

> Assay Mix from source plate Mix1 is transferred to all four quadrants of OLA reaction plate OLA1, Mix 2 to OLA2, Mix 3 to OLA3, and Mix 4 to OLA4.

In Method 1b_dry:

> Assay Mix from source plate Mix 1 is transferred to quadrant 1 of all four OLA reaction plates (OLA1 to OLA4), Mix 2 to quadrant 2, Mix 3 to quadrant 3, and Mix 4 to quadrant 4.

Preparing the Reagents

1. Thaw the SNPlex Oligonucleotide Ligation Kit components and probe pools at room temperature.
2. Label four skirted 96-well PCR plates Mix1 to Mix4.
3. Label four 15-mL centrifuge tubes Mix1 to Mix4.

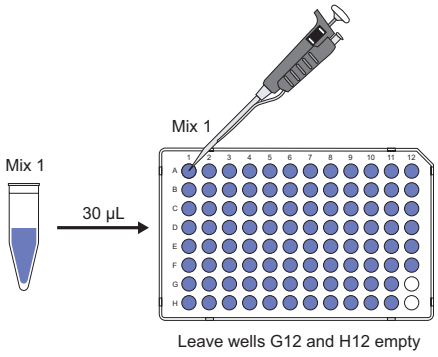
Notes

4. For each of the four SNPlex Ligation Probe Pools, prepare an Assay Mix in a 15-mL centrifuge tube and mix thoroughly.

	Manual	Automated		
		Automation Losses		Automation Totals
Reagent	**Volume (µL) for One Quadrant**	**Source Plate Dead Volume†**	**Transfer Loss Excess (µL)‡**	**Total Volume (µL) for Four Quadrants§**
Nuclease Free Water	216.2	432.4	110.4	1407.6
SNPlex™ OLA Master Mix	235.0	470.0	120.0	1530.0
SNPlex™ Universal Linkers, 48-plex	4.7	9.4	2.4	30.6
SNPlex™ dATP	4.7	9.4	2.4	30.6
SNPlex™Probe Pool (500 nM)	9.4	18.8	4.8	61.2
Total	470.0	940.0	240.0	3060.0

† 94 tips in source plate × 0µL per tip.
‡ Transfer losses for transferring bulk mixture into 96-well source plate.
§ Use the volumes in this column to prepare the reagents. Refer to the *SNPlex™ Genotyping System 48-plex User Guide* for per reaction volumes. Volumes given in this table include allowances for dead volume and excess volume for pipetting losses. For more information about calculating dead volumes, refer to the *SNPlex™ Genotyping System 48-plex General Automation Getting Started Guide*.

5. Add 30 µL of the Assay Mix into each well of a skirted 96-well PCR plate, excluding wells G12 and H12, then centrifuge the plate briefly to collect the contents at the bottom of the wells.

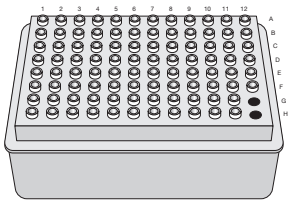


6. Label four 384-well plates, containing dried down gDNA samples, OLA1 to OLA4. (For information on the layout of dry gDNA plates, see Method "1a_dry" on page 21 or "1b_dry" on page 22.)

Notes

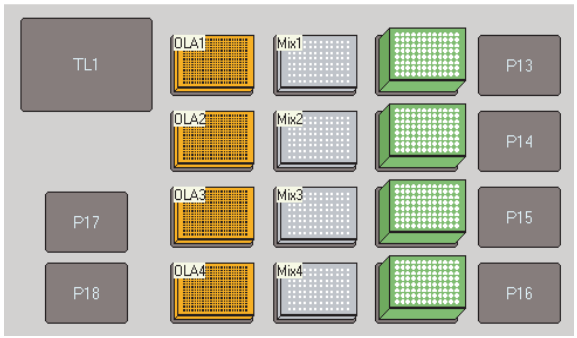
Running Methods 1a_dry and 1b_dry

1. Remove the tips corresponding to well positions G12 and H12 from every tip box.



Remove tips from wells G12 and H12

2. Place the uncovered tip boxes and plates on the deck as shown in the following figure, placing an ACME adapter underneath each OLA plate.

Plate	Position	Methods 1a_dry and 1b_dry Deck Layout 1
OLA1 to OLA4	P1 to P4	
Mix1 to Mix4	P5 to P8	
Uncovered tip box	P9 to P12	

3. Start the method using either:
   - 1a_SNPLEXv5_OLA_PN_S4_D.bmt for Method 1a_dry

   or
   - 1b_SNPLEXv5_OLA_P4_SN_D.bmt for Method 1b_dry

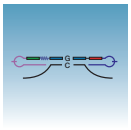
4. When the method pauses, verify that the deck layout corresponds to the figure above and click **OK** to continue.

5. After the method run is complete, remove and discard the tip boxes and plates Mix1 to Mix4.

6. Remove, seal, and briefly centrifuge the OLA reaction plates (OLA1 to OLA4).

7. Proceed to "Thermal Cycling the OLA Reactions" on page 27.

Notes



Thermal Cycling the OLA Reactions

Thermal cycle the OLA plates using the following conditions:

Step	Step Type	Temperature (°C)	Time
1	HOLD	48	30 min
2	HOLD	90	20 min
3	25 cycles	94	15 sec
		60	30 sec
		51, 3% ramp	30 sec
4	HOLD	99	10 min
5	HOLD	4	∞

Next Steps

At this point, the OLA reaction is complete. Proceed to Chapter 5, "Purifying OLA Products (Exonuclease)."

Notes

Performing the OLA Reactions: Biomek FX Method 1c_dry

About the Method

Method 1c_dry was developed for setups that have 92 gDNA samples and 16 ligation probe pools.

The method transfers Assay Mix from 16 plates into four OLA reaction plates in four steps, each corresponding to a deck layout:

- Assay Mix from source plates Mix1 to Mix4 is transferred to plate OLA1.
- Assay Mix from source plates Mix5 to Mix8 is transferred to plate OLA2.
- Assay Mix from source plates Mix9 to Mix12 is transferred to plate OLA3.
- Assay Mix from source plates Mix13 to Mix16 is transferred to plate OLA4.

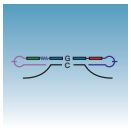
Preparing the Reagents

1. Thaw the SNPlex Oligonucleotide Ligation Kit components and probe pools at room temperature.
2. Label 16 skirted 96-well PCR plates Mix1 to Mix16.
3. Label 16 15-mL centrifuge tubes Mix1 to Mix16.
4. For each of the 16 SNPlex Ligation Probe Pools, prepare an Assay Mix in a 15-mL centrifuge tube and mix thoroughly.

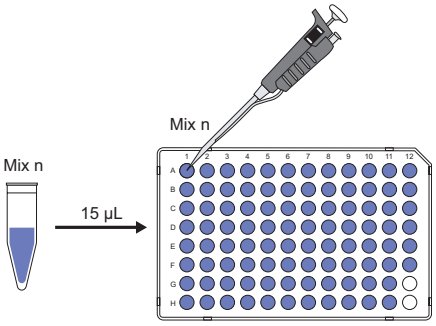
	Manual	Automated		
		Automation Losses		Automation Totals
Reagent	**Volume (µL) for One Quadrant**	**Source Plate Dead Volume†**	**Transfer Loss Excess (µL)‡**	**Total Volume (µL) for One Quadrant§**
Nuclease Free Water	216.2	432.4	110.4	759.0
SNPlex™ OLA Master Mix	235.0	470.0	120.0	825.0
SNPlex™ Universal Linkers, 48-plex	4.7	9.4	2.4	16.5
SNPlex™ dATP	4.7	9.4	2.4	16.5
SNPlex™Probe Pool (500 nM)	9.4	18.8	4.8	33.0
Total	470.0	940.0	240.0	1650.0

† 94 tips in source plate × 10µL per tip.
‡ Transfer losses for transferring bulk mixture into 96-well source plate.
§ Use the volumes in this column to prepare the reagents. Refer to the *SNPlex™ Genotyping System 48-plex User Guide* for per reaction volumes. Volumes given in this table include allowances for dead volume and excess volume for pipetting losses. For more information about calculating dead volumes, refer to the *SNPlex™ Genotyping System 48-plex General Automation Getting Started Guide*.

Notes



5. Add 15 µL of the Assay Mix into each well of a skirted 96-well PCR plate, excluding wells G12 and H12, then centrifuge the plate briefly to collect the contents at the bottom of the wells.

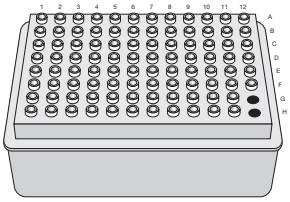


Leave wells G12 and H12 empty

6. Label four 384-well plates, containing dried gDNA, OLA1 to OLA4. (For information on the layout of dry gDNA plates, see Method "1c_dry" on page 22.)

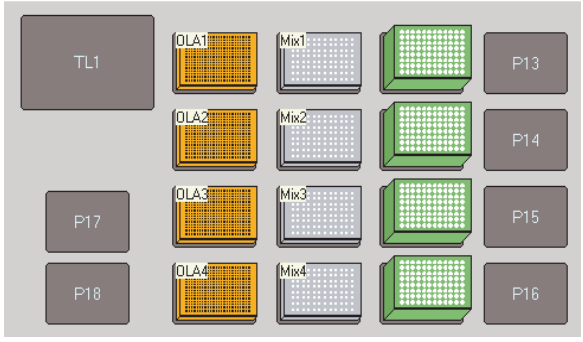
Running Method 1c_dry

1. Remove the tips corresponding to well positions G12 and H12 from every tip box.



Remove tips from wells G12 and H12

2. Place the uncovered tip boxes and plates on the deck as shown in the following figure, placing an ACME adapter underneath each OLA plate.

Plate	Position	Method 1c_dry Deck Layout 1
OLA1 to OLA4	P1 to P4	
Mix1 to Mix4	P5 to P8	
Uncovered tip box	P9 to P12	

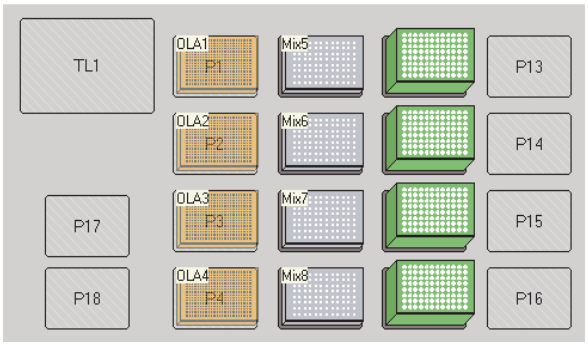
Notes

3. Start the method using 1c_SNPLEXv5_OLA_P16_S1_D.bmt.

4. When the method pauses, verify that the deck layout corresponds to the deck layout above and click **OK** to continue.

   The Biomek FX instrument transfers OLA mix from plates Mix1 to Mix4 to plate OLA1 and then pauses.

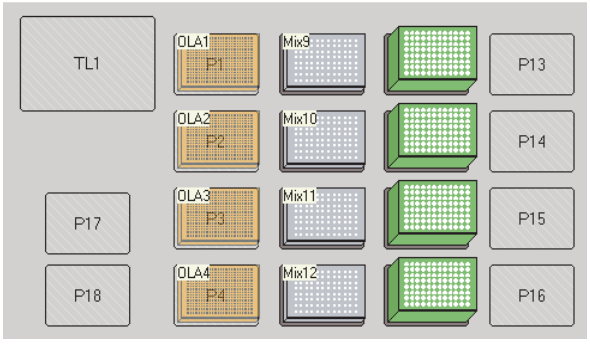
5. Remove the used tip boxes and plates Mix1 to Mix4, then replace the tip boxes and plates, leaving the OLA reaction plates in place, as specified in the following table:

Plate	Position	Method 1c_dry Deck Layout 2
OLA1 to OLA4	P1 to P4	
Mix5 to Mix8	P5 to P8	
Uncovered tip box	P9 to P12	

6. Click **OK** to continue.

   The Biomek FX instrument transfers OLA mix from plates Mix5 to Mix8 to plate OLA2 and then pauses.

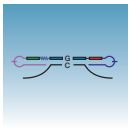
7. Remove the used tip boxes and plates Mix5 to Mix8, then replace the tip boxes and plates, leaving the OLA reaction plates in place, as specified in the following table:

Plate	Position	Method 1c_dry Deck Layout 3
OLA1 to OLA4	P1 to P4	
Mix9 to Mix12	P5 to P8	
Uncovered tip box	P9 to P12	

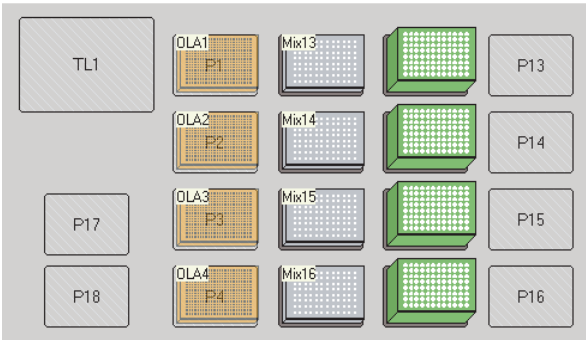
8. Click **OK** to continue.

   The Biomek FX instrument transfers OLA mix from plates Mix9 to Mix12 to plate OLA3 and then pauses.

Notes



9. Remove the used tip boxes and plates Mix9 to Mix12, then replace the tip boxes and plates, leaving the OLA reaction plates in place, as specified in the following table:

Plate	Position	Method 1c_dry Deck Layout 4
OLA1 to OLA4	P1 to P4	
Mix13 to Mix16	P5 to P8	
Uncovered tip box	P9 to P12	

10. Click **OK** to continue.

    The Biomek FX instrument transfers OLA mix from plates Mix13 to Mix16 to plate OLA4.

11. After the method run is complete, remove and discard the tip boxes and plates (Mix13 to Mix16) and remove, seal, and briefly centrifuge the OLA reaction plates (OLA1 to OLA4).

12. Proceed to "Thermal Cycling the OLA Reactions."

Thermal Cycling the OLA Reactions

Thermal cycle the OLA plates using the following conditions:

Step	Step Type	Temperature (°C)	Time
1	HOLD	48	30 min
2	HOLD	90	20 min
3	25 cycles	94	15 sec
		60	30 sec
		51, 3% ramp	30 sec
4	HOLD	99	10 min
5	HOLD	4	∞

Next Steps

At this point, the OLA reaction is complete. Proceed to Chapter 5, "Purifying OLA Products (Exonuclease)."

Notes

Performing the OLA Reactions: Biomek FX Method 1d_dry

About These Methods

Method 1d_dry was developed for setups that have 1472 gDNA samples and 1 ligation probe pool and uses use a single deck layout.

To minimize tip usage and prevent carryover contamination between quadrants, these methods are designed so that the tips are positioned above the liquid level in the plates when dispensing reagents.

Preparing the Reagents

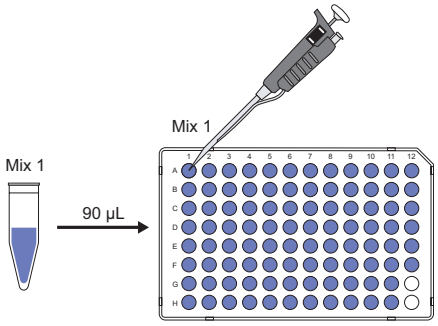
1. Thaw the SNPlex Oligonucleotide Ligation Kit components and probe pool at room temperature.
2. Label a skirted 96-well PCR plate Assay Mix.
3. Prepare the Assay Mix in a 15-mL centrifuge tube and mix thoroughly.

	Manual	Automated		
		Automation Losses		Automation Totals
Reagent	**Volume (µL) for One Quadrant**	**Source Plate Dead Volume†**	**Transfer Loss Excess (µL)‡**	**Total Volume (µL) for 16 Quadrant§**
Nuclease Free Water	216.2	432.4	110.4	4002.0
SNPlex™ OLA Master Mix	235.0	470.0	120.0	4350.0
SNPlex™ Universal Linkers, 48-plex	4.7	9.4	2.4	87.0
SNPlex™ dATP	4.7	9.4	2.4	87.0
SNPlex™Probe Pool (500 nM)	9.4	18.8	4.8	174.0
Total	470.0	940.0	240.0	8700.0

† 94 tips in source plate × 10µL per tip.
‡ Transfer losses for transferring bulk mixture into 96-well source plate.
§ Use the volumes in this column to prepare the reagents. Refer to the *SNPlex™ Genotyping System 48-plex User Guide* for per reaction volumes. Volumes given in this table include allowances for dead volume and excess volume for pipetting losses. For more information about calculating dead volumes, refer to the *SNPlex™ Genotyping System 48-plex General Automation Getting Started Guide*.

Notes

4. Add 90 µL of the Assay Mix into each well of a skirted 96-well PCR plate, excluding wells G12 and H12, then centrifuge the plate briefly to collect the contents at the bottom of the wells.

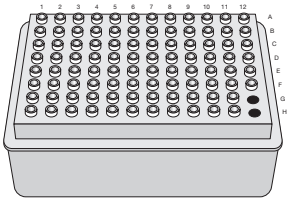


Leave wells G12 and H12 empty

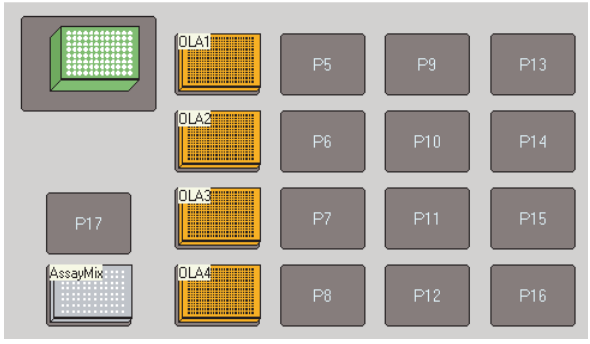
5. Label four 384-well plates, containing dried DNA, OLA1 to OLA4. (For information on the layout of dry gDNA plates, see Method "1d_dry" on page 23.)

Running Method 1d_dry

1. Remove the tips corresponding to well positions G12 and H12 from every tip box.


Remove tips from wells G12 and H12

2. Place the uncovered tip boxes and plates on the deck as shown in the following figure, placing an ACME adapter underneath each OLA plate.

Plate	Position	Method1d_dry Deck Layout 1
Uncovered tip box	TL1	
AssayMix	P18	
OLA1 to OLA4	P1 to P4	

3. Start the method using 1d_SNPLEXv5_OLA_P1_S16_D.bmt.

4. When the method pauses, verify that the deck layout corresponds to the deck layout above and click **OK** to continue.

Notes

5. After the method run is complete, remove and discard the tip boxes and the Assay Mix plate and remove, seal, and briefly centrifuge the OLA reaction plates (OLA1 to OLA4).

6. Proceed to "Thermal Cycling the OLA Reactions."

Thermal Cycling the OLA Reactions

Thermal cycle the OLA plates using the following conditions:

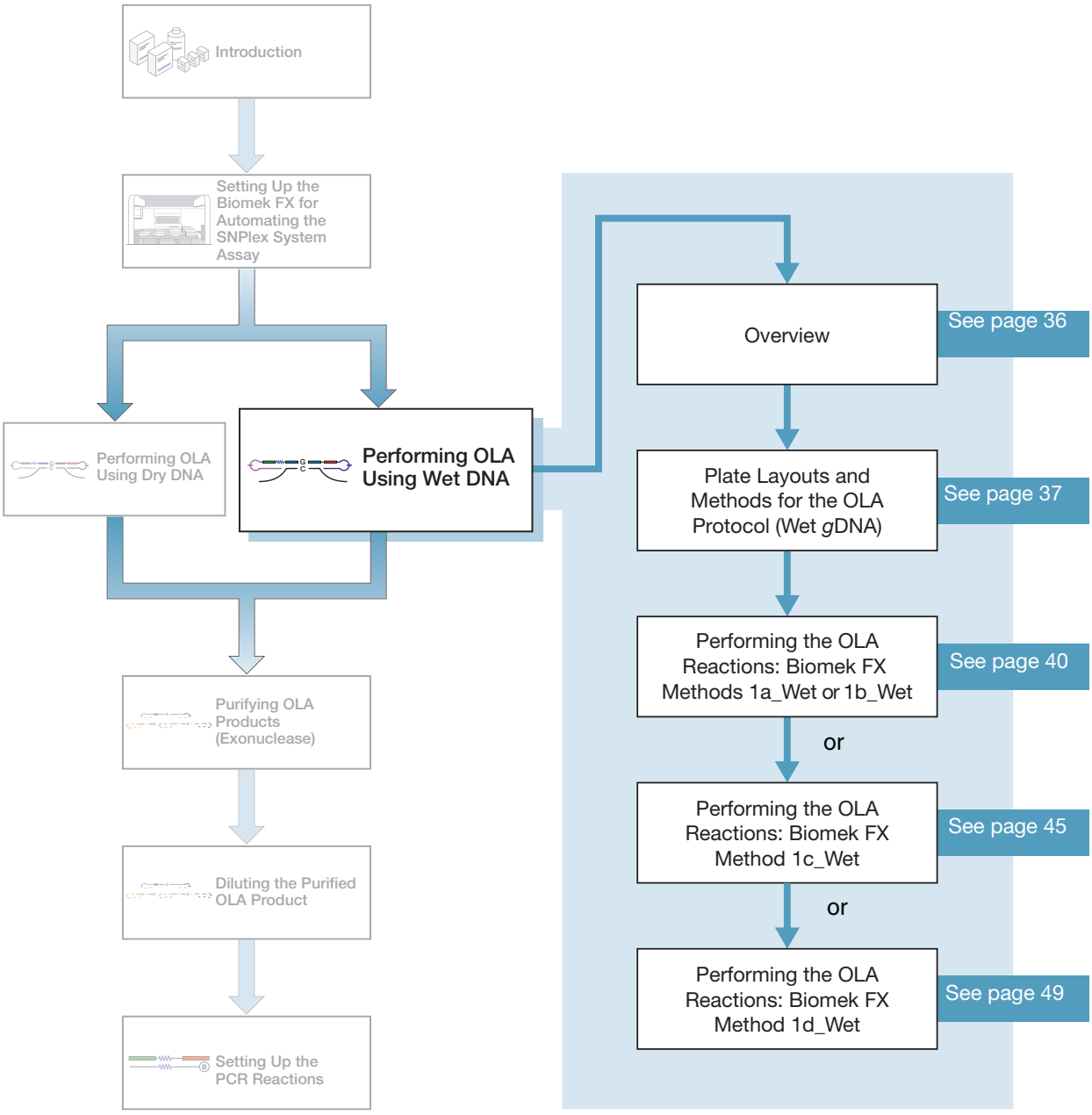
Step	Step Type	Temperature (°C)	Time
1	HOLD	48	30 min
2	HOLD	90	20 min
3	25 cycles	94	15 sec
		60	30 sec
		51, 3% ramp	30 sec
4	HOLD	99	10 min
5	HOLD	4	∞

Next Steps

At this point, the OLA reaction is complete. Proceed to Chapter 5, "Purifying OLA Products (Exonuclease)."

Notes

Performing OLA Using Wet gDNA


Introduction
Setting Up the Biomek FX for Automating the SNPlex System Assay
Performing OLA Using Dry DNA
Performing OLA Using Wet DNA
Purifying OLA Products (Exonuclease)
Diluting the Purified OLA Product
Setting Up the PCR Reactions
Overview
See page 36
Plate Layouts and Methods for the OLA Protocol (Wet gDNA)
See page 37
Performing the OLA Reactions: Biomek FX Methods 1a_Wet or 1b_Wet
See page 40
or
Performing the OLA Reactions: Biomek FX Method 1c_Wet
See page 45
or
Performing the OLA Reactions: Biomek FX Method 1d_Wet
See page 49

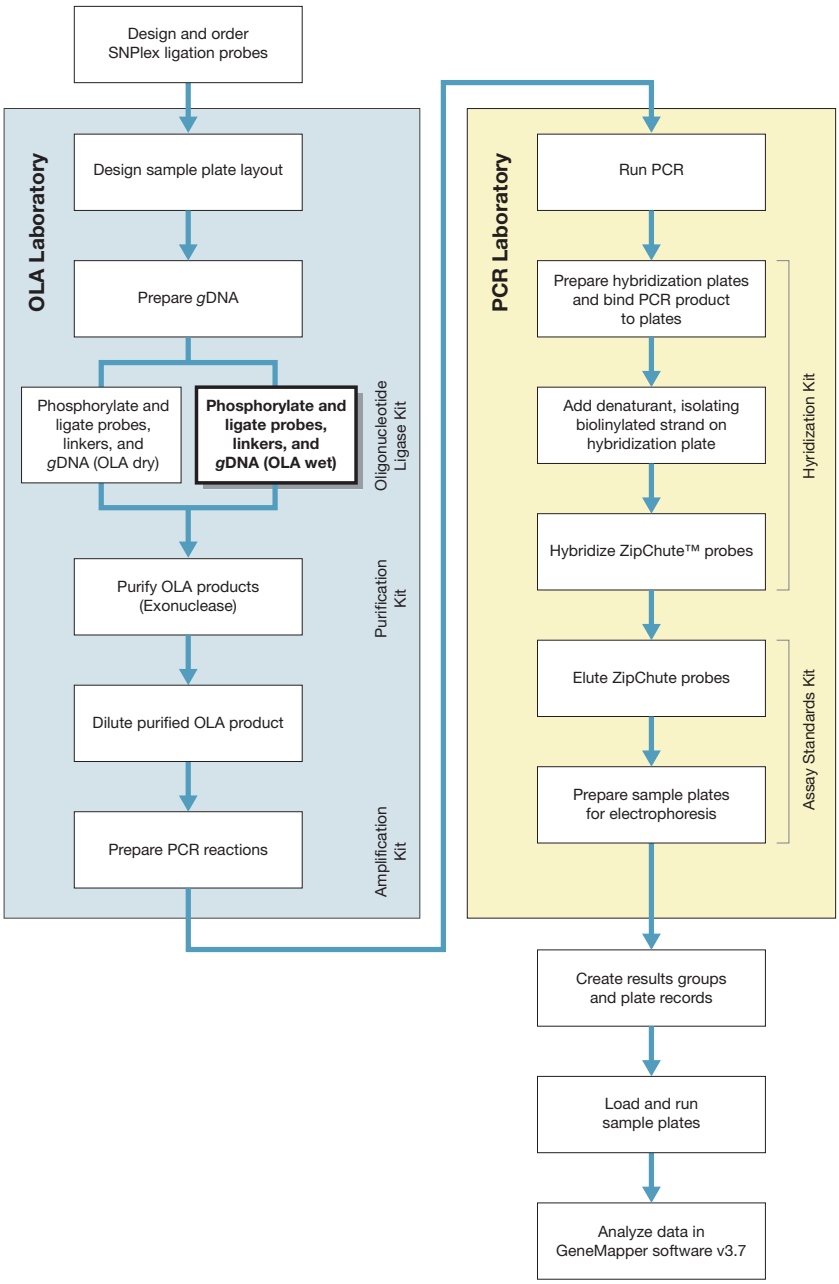
Notes

Overview

About This Chapter

This chapter provides information about automating the OLA protocol with the Biomek FX with disposable tips. The procedures in this chapter assume the use of a batch of four SNPlex™ OLA reaction plates, and that the gDNA samples have been quantified, fragmented, and diluted to a concentration of 18.5 ng/µL before beginning this procedure. If you are using dry gDNA, refer to Chapter 3, "Performing OLA Using Dry gDNA."

Where You Are In the SNPlex System Assay Workflow


Design and order SNPlex ligation probes
OLA Laboratory
Design sample plate layout
Prepare gDNA
Phosphorylate and ligate probes, linkers, and gDNA (OLA dry)
Phosphorylate and ligate probes, linkers, and gDNA (OLA wet)
Oligonucleotide Ligase Kit
Purify OLA products (Exonuclease)
Purification Kit
Dilute purified OLA product
Prepare PCR reactions
Amplification Kit
PCR Laboratory
Run PCR
Prepare hybridization plates and bind PCR product to plates
Add denaturant, isolating biolinylated strand on hybridization plate
Hybridize ZipChute™ probes
Hyridization Kit
Elute ZipChute probes
Prepare sample plates for electrophoresis
Assay Standards Kit
Create results groups and plate records
Load and run sample plates
Analyze data in GeneMapper software v3.7

Notes

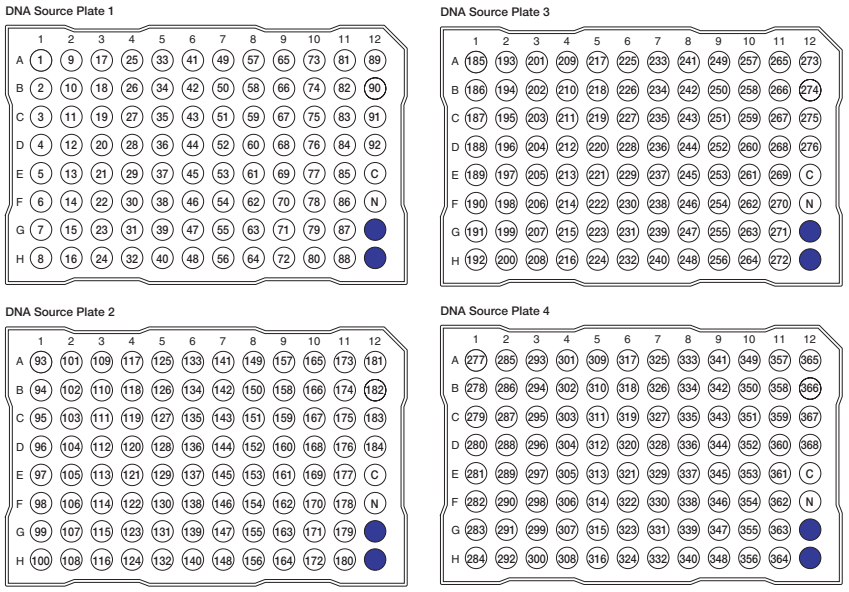
Plate Layouts and Methods for the OLA Protocol (Wet gDNA)

You can select from four Biomek FX methods depending on the number of samples and probe pools assayed in a single run.

Arraying gDNA in 96-Well Plates

Array your gDNA samples in skirted 96-well PCR plates, 92 samples per plate. Reserve well E12 for the SNPlex System kit control gDNA and well F12 for the No Template Control (NTC). Leave wells G12 and H12 empty - these wells are used as Allelic Ladder wells during analysis.

For example, you may array 368 gDNA samples among four 96-well plates as shown:



Volumes for Source Plates

The volumes of the gDNA samples you use depend on your total number of samples and the number of SNPlex Ligation Probe pools you are using. The volumes in the table below include dead volumes to be sure the robotic tips remain submerged during aspiration.

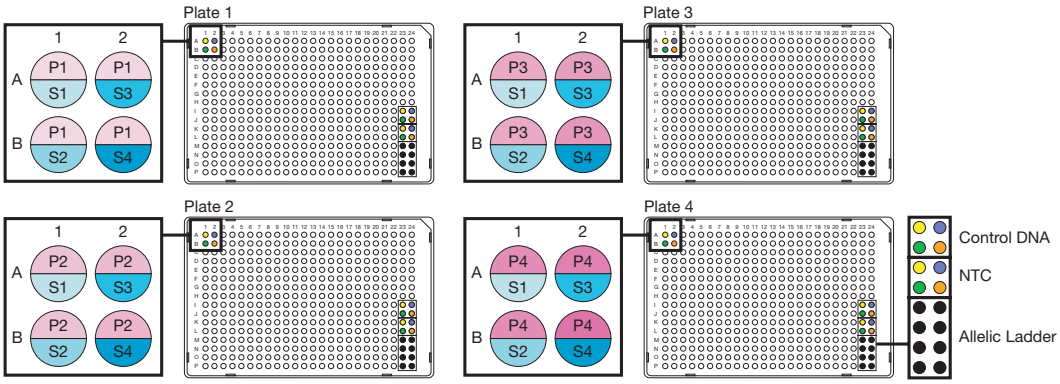
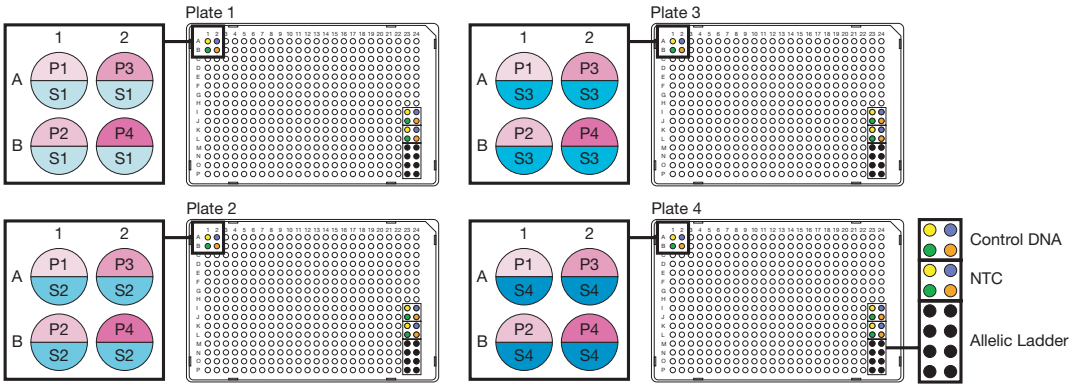
Method Number	Method File Name	Number of Probe Pools	Number of gDNA Source Plates	Total Number of gDNA Samples	Required Volume (µL) of gDNA Samples
1a_wet	1a_SNPLEXv5_OLA_PN_S4_W.bmt	4	4	368	20
1b_wet	1b_SNPLEXv5_OLA_P4_SN_W.bmt	4	4	368	20
1c_wet	1c_SNPLEXv5_OLA_P16_S1_W.bmt	16	1	92	40
1d_wet	1d_SNPLEXv5_OLA_P1_S16_W.bmt	1	16	1472	12

Notes

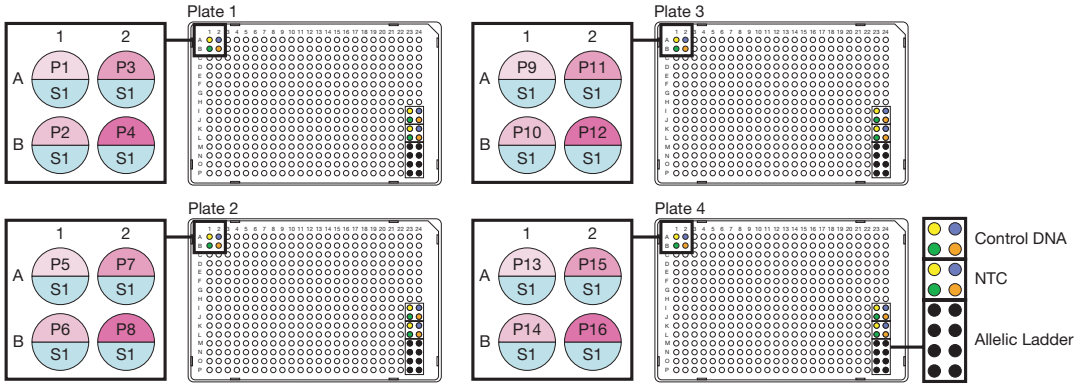
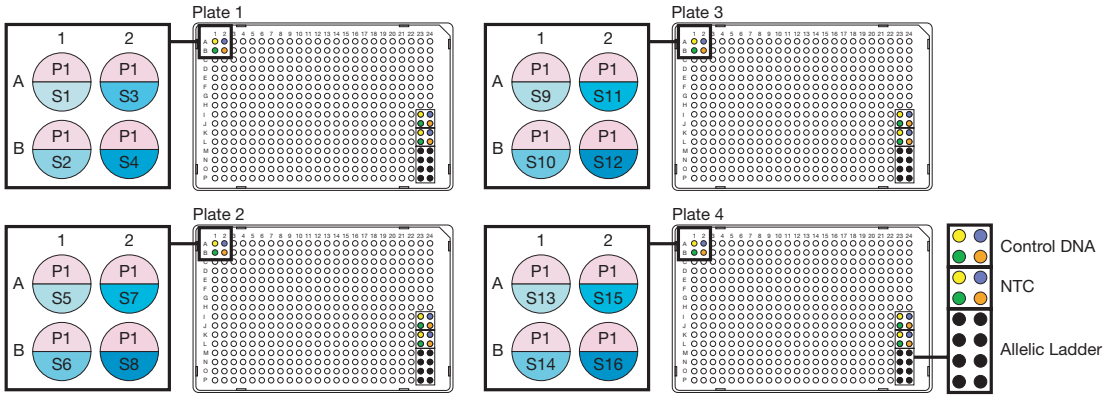
Reaction Plate Layouts

You can select from four Biomek FX methods depending on the number of samples and probe pools assayed in a single run. The following table gives the plate layouts and the associated file for each layout. The quadrant representation comes from the division of the 384-well reaction plates into four 96-well quadrants.

Although plate layouts are flexible, some layouts are more efficient with reagent usage than others. The layout that assays 1472 samples with a single probe pool is most efficient since reagent dead volume is limited to a single 96-well source container. The layout that assays 92 samples with 16 probe pools is the most inefficient since reagent dead volume is spread across sixteen 96-well source containers. For optimal use of SNPlex System reagent kits, consider plate layouts, batch sizes, and "dried vs wet" gDNA setup.

Method Number†	Method File Name‡	Number of Probe Pools	Total Number of Samples§	Reference
1a_wet	1a_SNPLEXv5_OLA_PN_S4_W.bmt	4	368	"Performing the OLA Reactions: Biomek FX Methods 1a_wet or 1b_wet" on page 40
				
1b_wet	1b_SNPLEXv5_OLA_P4_SN_W.bmt	4	368	"Performing the OLA Reactions: Biomek FX Methods 1a_wet or 1b_wet" on page 40
				

Notes

Method Number†	Method File Name‡	Number of Probe Pools	Total Number of Samples§	Reference
1c_wet	1c_SNPLEXv5_OLA_P16_S1_W.bmt	16	92	"Performing the OLA Reactions: Biomek FX Method 1c_wet" on page 45
				
1d_wet	1d_SNPLEXv5_OLA_P1_S16_W.bmt	1	1472	"Performing the OLA Reactions: Method 1d_wet" on page 49
				

† Method numbering reflects methods provided for other SNPlex System assay protocols. Methods 1a_dry through 1d_dry in this Guide are described in Chapter 3, "Performing OLA Using Dry gDNA.".

‡ P = Probe Pool; S = Unique collection of 92 gDNA samples (4 positions of a 96-well DNA source plate are reserved for 1 control DNA, 1 NTC, and 2 allelic ladder wells)

§ Total number of samples in a batch containing four 384-well plates. Does not include control DNA or NTC.

Notes

Performing the OLA Reactions: Biomek FX Methods 1a_wet or 1b_wet

About These Methods

Methods 1a_wet and 1b_wet were developed for setups that have 368 gDNA samples and four probe pools.

These methods use identical three-deck layouts. The difference between them is the pattern in which the instrument dispenses the reagents and DNA samples into the wells of the OLA reaction plates.

In Method 1a_wet:

- Assay Mix from source plate Mix1 is transferred to all four quadrants of OLA reaction plate OLA1, Mix 2 to OLA2, Mix 3 to OLA3, and Mix 4 to OLA4.
- Samples from source plate DNA1 are transferred to quadrant 1 of all four OLA plates (OLA1 to OLA4), DNA2 to quadrant 2, DNA3 to quadrant 3, and DNA4 to quadrant 4.

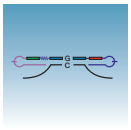
In Method 1b_wet:

- Assay Mix from source plate Mix 1 is transferred to quadrant 1 of all four OLA reaction plates (OLA1 to OLA4), Mix 2 to quadrant 2, Mix 3 to quadrant 3, and Mix 4 to quadrant 4.
- Samples from source plate DNA1 are transferred to all wells of plate OLA1, DNA2 to OLA2, DNA3 to OLA3, and DNA4 to OLA4.

Preparing the Reagents

1. Thaw the SNPlex Oligonucleotide Ligation Kit components and probe pools at room temperature.
2. Label four skirted 96-well PCR plates Mix1 to Mix4.
3. Label four 15-mL centrifuge tubes Mix1 to Mix4.
4. Label four empty 96-well plates for use as DNA source plates DNA1 to DNA4. (For more information on DNA source plate layouts, see "Plate Layouts and Methods for the OLA Protocol (Wet gDNA)" on page 37).

Notes

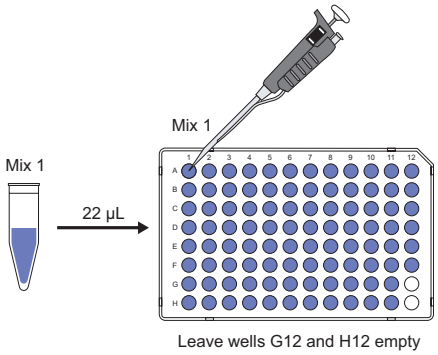


5. For each of the four SNPlex Ligation Probe Pools, prepare an Assay Mix in a 15-mL centrifuge tube and mix thoroughly.

	Manual	Automated		
		Automation Losses		Automation Totals
Reagent	**Volume (µL) for One Quadrant**	**Source Plate Dead Volume†**	**Transfer Loss Excess (µL)‡**	**Total Volume (µL) for Four Quadrants§**
Nuclease Free Water	28.2	94.0	21.8	228.6
SNPlex™ OLA Master Mix	235.0	783.3	181.7	1905.0
SNPlex™ Universal Linkers, 48-plex	4.7	15.7	3.6	38.1
SNPlex™ dATP	4.7	15.7	3.6	38.1
SNPlex™Probe Pool (500 nM)	9.4	31.3	7.3	76.2
Total	282.0	940.0	218.0	2286.0

† 94 tips in source plate × 10µL per tip.
‡ Transfer losses for transferring bulk mixture into 96-well source plate.
§ Use the volumes in this column to prepare the reagents. Refer to the *SNPlex™ Genotyping System 48-plex User Guide* for per reaction volumes. Volumes given in this table include allowances for dead volume and excess volume for pipetting losses. For more information about calculating dead volumes, refer to the *SNPlex™ Genotyping System 48-plex General Automation Getting Started Guide*.

6. Add 22 µL of the Assay Mix into each well of a skirted 96-well PCR plate, excluding wells G12 and H12, then centrifuge the plate briefly to collect the contents at the bottom of the wells.

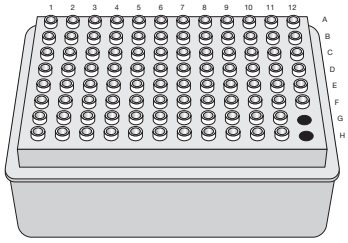


7. Label four 384-well ABI clear optical reaction plates OLA1 to OLA4. (For information on the layout of wet gDNA plates, see Method "1a_wet," or "1b_wet" on page 37.)

Notes

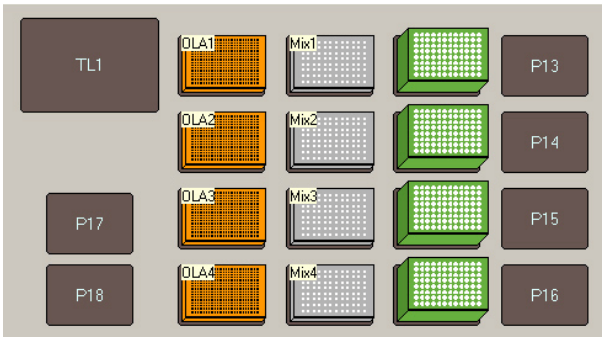
Running Method 1a_wet or 1b_wet

1. Remove the tips corresponding to well positions G12 and H12 from every tip box.



Remove tips from wells G12 and H12

2. Place the uncovered tip boxes and plates on the deck as shown in the following figure, placing an ACME adapter underneath each OLA plate.

Plate	Position	Methods 1a_wet and 1b_wet, Deck Layout 1
OLA1 to OLA4	P1 to P4	
Mix1 to Mix4	P5 to P8	
Uncovered tip box	P9 to P12	

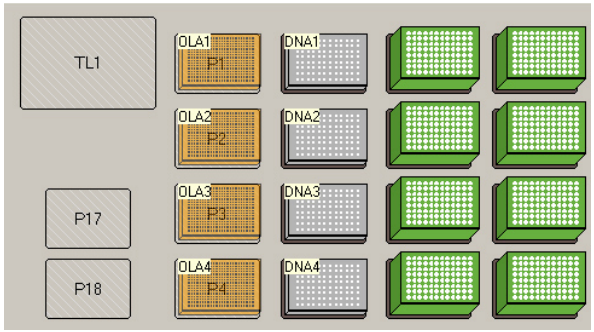
3. Start the method using either:
   - 1a_SNPLEXv5_OLA_PN_S4_W.bmt for Method 1a_wet

   or
   - 1b_SNPLEXv5_OLA_P4_SN_W.bmt for Method 1b_wet

   When the method pauses, verify that the deck layout corresponds to the figure above and select **OK** to continue.

Notes

4. When the method pauses, remove and discard the tip boxes and plates Mix1 to Mix4. Remove the tips corresponding to well positons G12 and H12 from eight new uncovered tip boxes and place them the DNA sample plates on the deck as shown in the deck layout below

Plate	Position	Methods 1a_wet and 1b_wet, Deck Layout 2 and 3
OLA1 to OLA4	P1 to P4	
DNA1 to DNA4	P5 to P8	
Uncovered tip box	P9 to P16	

5. Click **OK** to continue the method.

6. When the method pauses, remove the tips corresponding to well positions G12 and H12 from eight new uncovered tip boxes and replace the used tip boxes in positions P9 to P16.

7. Click **OK** to continue the method.

8. After the method completes, remove, seal, and store (if necessary) the DNA sample plates (DNA1 to DNA4).

9. Remove, seal and briefly centrifuge the OLA reaction plates (OLA1 to OLA4).

10. Proceed to "Thermal Cycling the OLA Reactions" on page 44.

Notes

Thermal Cycling the OLA Reactions

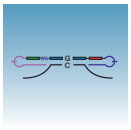
Thermal cycle the OLA plates using the following conditions:

Step	Step Type	Temperature (°C)	Time
1	HOLD	48	30 min
2	HOLD	90	20 min
3	25 cycles	94	15 sec
		60	30 sec
		51, 3% ramp	30 sec
4	HOLD	99	10 min
5	HOLD	4	∞

Next Steps

At this point, the OLA reaction is complete. Proceed to Chapter 5, "Purifying OLA Products (Exonuclease)."

Notes



Performing the OLA Reactions: Biomek FX Method 1c_wet

About the Method

Method 1c_wet was developed for setups that have 92 gDNA samples and 16 ligation probe pools.

The method transfers DNA samples and 16 Assay Mixes into four OLA reaction plates in four steps, each corresponding to a deck layout:

- DNA samples are transferred to all OLA plates, then Assay Mix from source plates Mix1 to Mix4 is transferred to plate OLA1.
- Assay Mix from source plates Mix5 to Mix8 is transferred to plate OLA2.
- Assay Mix from source plates Mix9 to Mix12 is transferred to plate OLA3.
- Assay Mix from source plates Mix13 to Mix16 is transferred to plate OLA4.

Preparing the Reagents

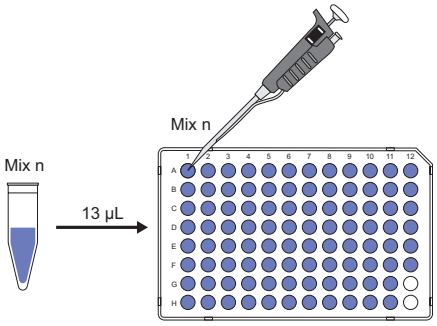
1. Label 16 skirted 96-well PCR plates Mix1 to Mix16.
2. Label 16 15-mL centrifuge tubes Mix1 to Mix16.
3. Label a DNA source plate DNA Sample. (For information on setting up DNA source plates, see "Arraying gDNA in 96-Well Plates" on page 37.)
4. For each of the 16 SNPlex Ligation Probe Pools, prepare an Assay Mix in a 15-mL centrifuge tube and mix thoroughly.

	Manual	Automated		
		Automation Losses		Automation Totals
Reagent	**Volume (µL) for One Quadrant**	**Source Plate Dead Volume†**	**Transfer Loss Excess (µL)‡**	**Total Volume (µL) for One Quadrant§**
Nuclease Free Water	28.2	94.0	21.8	144.0
SNPlex™ OLA Master Mix	235.0	783.3	181.7	1200.0
SNPlex™ Universal Linkers, 48-plex	4.7	15.7	3.6	24.0
SNPlex™ dATP	4.7	15.7	3.6	24.0
SNPlex™Probe Pool (500 nM)	9.4	31.3	7.3	48.0
Total	282.0	940.0	218.0	1440.0

† 94 tips in source plate × 10µL per tip.
‡ Transfer losses for transferring bulk mixture into 96-well source plate.
§ Use the volumes in this column to prepare the reagents. Refer to the *SNPlex™ Genotyping System 48-plex User Guide* for per reaction volumes. Volumes given in this table include allowances for dead volume and excess volume for pipetting losses. For more information about calculating dead volumes, refer to the *SNPlex™ Genotyping System 48-plex General Automation Getting Started Guide*.

Notes

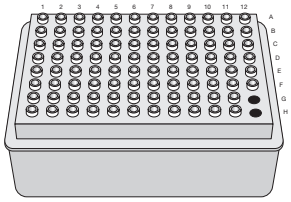
5. Add 13 µL of the Assay Mix into each well of a skirted 96-well PCR plate, excluding wells G12 and H12, then centrifuge the plate briefly to collect the contents at the bottom of the wells.



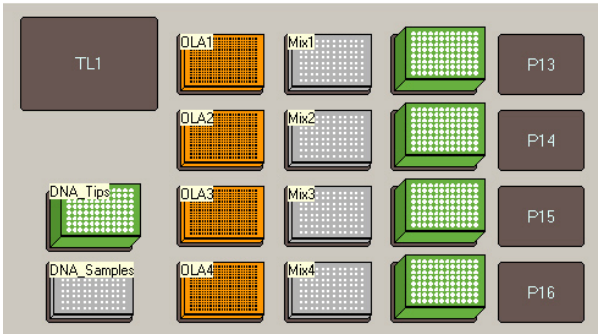
6. Label four 384-well clear optical reaction plates OLA1 to OLA4. (For information on the layout of wet gDNA plates, see Method "1c_wet" on page 37.)

Running Method 1c_wet

1. Remove the tips corresponding to well positions G12 and H12 from every tip box.



2. Place the five uncovered tip boxes and plates on the deck as shown in the deck layout below..

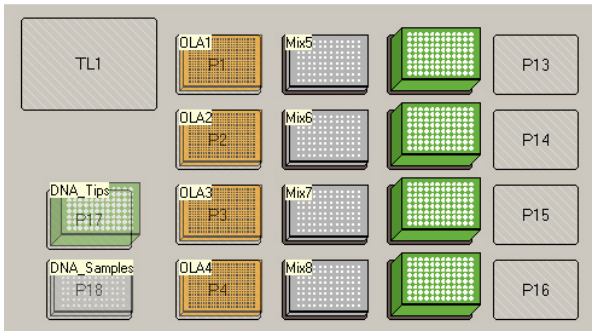
Plate	Position	Method 1c_wet Deck Layout 1
OLA1 to OLA4	P1 to P4	
DNA sample	P18	
Mix1 to Mix4	P5 to P8	
Uncovered tip box	P9to P12, P17	

3. Start the method using 1c_SNPLEXv5_OLA_P16_S1_W.bmt.

4. When the method pauses, remove and discard the four used tip boxes (from P9 to P12) and plates Mix1 to Mix4 (from P5 to P8).

Notes

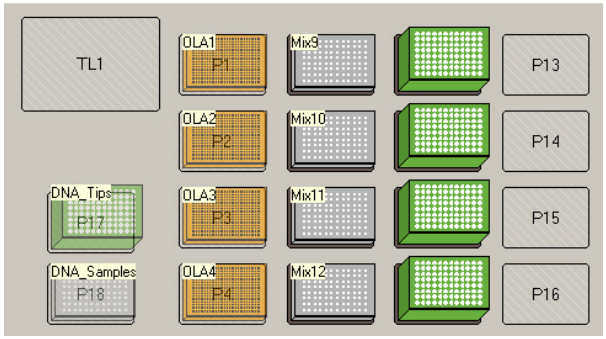
5. Remove the tips corresponding to well positions G12 and H12 from four new tip boxes and place them and the plates as shown in the deck layout below.

Plate	Position	Method 1c_wet Deck Layout 2
OLA1 to OLA4	P1 to P4	
DNA sample	P18	
Mix5 to Mix8	P5 to P8	
Uncovered tip box	P9 to P12	

6. Click **OK** to continue the method.

7. When the method pauses, remove the four used tip boxes in positions P9 to P12 and the Assay Mix plates (Mix5 to Mix8) from positions P5 to P8.

8. Remove the tips corresponding to well positions G12 and H12 from four new tip boxes and place them and the plates as shown in the deck layout below.

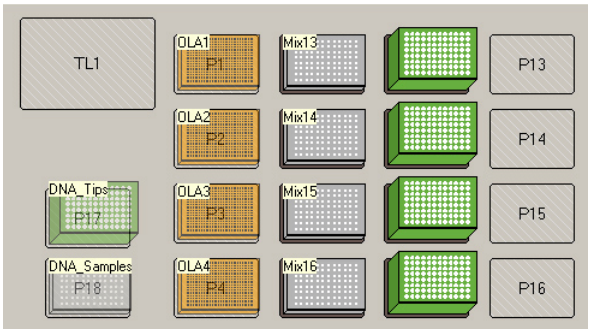
Plate	Position	Method 1c_wet Deck Layout 3
OLA1 to OLA4	P1 to P4	
DNA sample	P18	
Mix9 to Mix12	P5 to P8	
Uncovered tip box	P9 to P12	

9. Click **OK** to continue the method.

10. When the method pauses, remove the four used tip boxes in positions P9 to P12 and the Assay Mix plates (Mix9 to Mix12) from positions P5 to P8.

Notes

11. Remove the tips corresponding to well positions G12 and H12 from four new tip boxes and place them and the plates as shown in the deck layout below.

Plate	Position	Method 1c_wet Deck Layout 4
OLA1 to OLA4	P1 to P4	
DNA sample	P18	
Mix13 to Mix16	P5 to P8	
Uncovered tip box	P9 to P12	

12. Click **OK** to continue the method.

13. After the method run is complete, remove and discard the tip boxes and plates (Mix13 to Mix16) and remove, seal, and store (if necessary) the DNA Samples plate.

14. Remove, seal, and briefly centrifuge the OLA reaction plates (OLA1 to OLA4).

15. Proceed to "Thermal Cycling the OLA Reactions."

Thermal Cycling the OLA Reactions

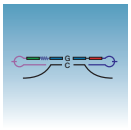
Thermal cycle the OLA plates using the following conditions:

Step	Step Type	Temperature (°C)	Time
1	HOLD	48	30 min
2	HOLD	90	20 min
3	25 cycles	94	15 sec
		60	30 sec
		51, 3% ramp	30 sec
4	HOLD	99	10 min
5	HOLD	4	∞

Next Steps

At this point, the OLA reaction is complete. Proceed to Chapter 5, "Purifying OLA Products (Exonuclease)."

Notes



Performing the OLA Reactions: Method 1d_wet

About The Method

Method 1d_wet was developed for setups that have 1472 gDNA samples and 1 ligation probe pool and uses four deck layouts:

- Assay Mix is added to all OLA plates, then DNA samples from source plates DNA1 to DNA4) are transferred to plate OLA1.
- DNA samples from source plates DNA5 to DNA8 are transferred to plate OLA2.
- DNA samples from source plates DNA9 to DNA12 are transferred to plate OLA3.
- DNA samples from source plates DNA13 to DNA16 are transferred to OLA4.

Preparing the Reagents

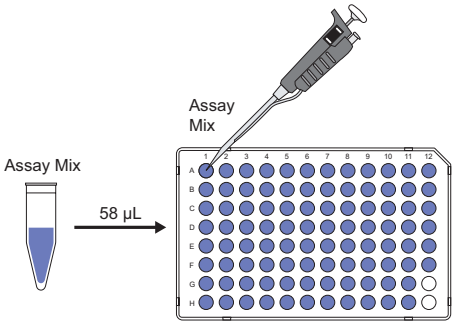
1. Label a skirted 96-well PCR plate Assay Mix.
2. Label 16 wet DNA source plates DNA1 to DNA16. (For information on setting up DNA source plates, see "Arraying gDNA in 96-Well Plates" on page 37.)
3. Prepare the Assay Mix in a 15-mL centrifuge tube and mix thoroughly..

	Manual	Automated		
		Automation Losses		Automation Totals
Reagent	**Volume (µL) for One Quadrant**	**Source Plate Dead Volume†**	**Transfer Loss Excess (µL)‡**	**Total Volume (µL) for Sixteen Quadrants§**
Nuclease Free Water	28.2	94.0	21.8	567.0
SNPlex™ OLA Master Mix	235.0	783.3	181.7	4725.0
SNPlex™ Universal Linkers, 48-plex	4.7	15.7	3.6	94.5
SNPlex™ dATP	4.7	15.7	3.6	94.5
SNPlex™Probe Pool (500 nM)	9.4	31.3	7.3	189.0
Total	282.0	940.0	218.0	5670.0

† 94 tips in source plate × 10µL per tip
‡ Transfer pipetting losses for transferring bulk mixture into 96-well source plate
§ Use the volumes in this column to prepare the reagents. Refer to the *SNPlex™ Genotyping System 48-plex User Guide* for per reaction volumes. Volumes given in this table include allowances for dead volume and excess volume for pipetting losses. For more information about calculating dead volumes, refer to the *SNPlex™ Genotyping System 48-plex General Automation Getting Started Guide*.

Notes

4. Add 58 µL of the Assay Mix into each well of a skirted 96-well PCR plate, excluding wells G12 and H12, then centrifuge the plate briefly to collect the contents at the bottom of the wells.

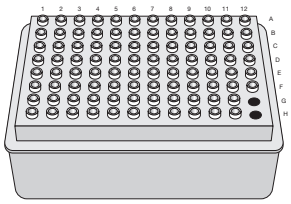


Leave wells G12 and H12 empty

5. Label four 384-well ABI PRISM® clear optical reaction plates OLA1 to OLA4. (For information on the layout of wet gDNA plates, see Method "1d_wet" on page 37.)

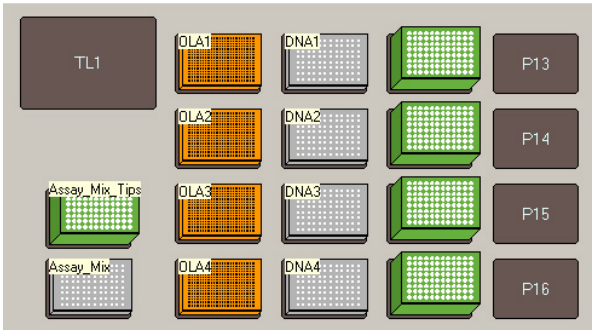
Running Method 1d_wet

1. Remove the tips corresponding to well positions G12 and H12 from five tip boxes.



Remove tips from wells G12 and H12

2. Place the five uncovered tip boxes and the plates on the deck as shown in the deck layout below.

Plate	Position	Method 1d_wet Deck Layout 1
OLA1 to OLA4	P1 to P4	
Assay Mix	P18	
DNA1 to DNA4	P5 to P8	
Uncovered tip box	P9to P12, P17	

3. Start the method using 1d_SNPLEXv5_OLA_P1_S16_W.bmt.

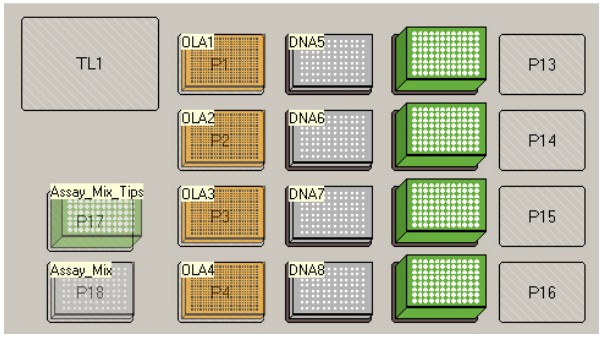
Notes

4. When the method pauses, click **OK** to continue.

5. When the method pauses, remove and discard the four used tip boxes (from P9 to P12).

6. Seal and store, if necessary, the DNA sample plates (DNA1 to DNA4) from positions P5 to P8.

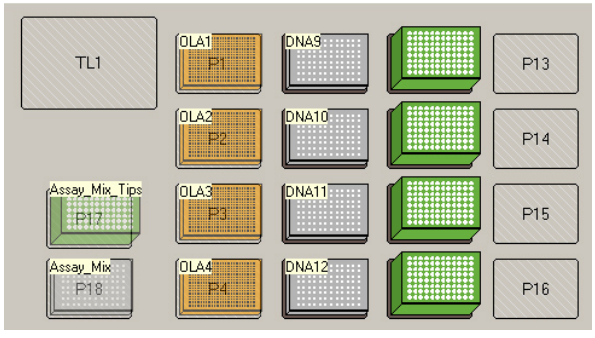
7. Remove the tips corresponding to well positions G12 and H12 from four new uncovered tip boxes and place them and the plates on the deck as shown in the deck layout below.

Plate	Position	Method 1d_wet Deck Layout 2
OLA1 to OLA4	P1 to P4	
Assay Mix	P18	
DNA5 to DNA8	P5 to P8	
Uncovered tip box	P9 to P12	

8. When the method pauses, click **OK** to continue the method.

9. When the method pauses, remove the four used tip boxes in positions P9 to P12 and the DNA sample plates (DNA5 to DNA8) from positions P5 to P8.

10. Remove the tips corresponding to well positions G12 and H12 from four new uncovered tip boxes and place them and the plates on the deck as shown in the deck layout below.

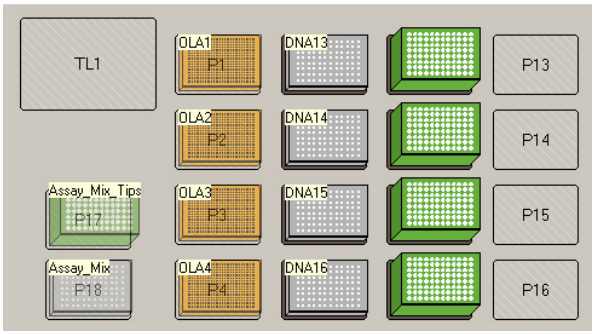
Plate	Position	Method 1d_wet Deck Layout 3
OLA1 to OLA4	P1 to P4	
Assay Mix	P18	
DNA9 to DNA12	P5 to P8	
Uncovered tip box	P9 to P12	

11. Click **OK** to continue the method.

Notes

12. When the method pauses, remove the four used tip boxes in positions P9 to P12 and the DNA sample plates (DNA9 to DNA12) from positions P5 to P8.

13. Remove the tips corresponding to well positions G12 and H12 from four new uncovered tip boxes and place them and the plates on the deck as shown in the deck layout below.

Plate	Position	Method 1d_wet Deck Layout 4
OLA1 to OLA4	P1 to P4	
Assay Mix	P18	
DNA13 to DNA16	P5 to P8	
Uncovered tip box	P9 to P12	

14. Click **OK** to continue the method.

15. After the method run is complete, remove and discard the tip boxes.
    - Seal and store plates DNA13 to DNA16, if necessary.
    - Remove the Assay Mix plate and discard.
    - Remove, seal, and briefly centrifuge the OLA reaction plates (OLA1 to OLA4).

16. Proceed to "Thermal Cycling the OLA Reactions."

Thermal Cycling the OLA Reactions

Thermal cycle the OLA plates using the following conditions:

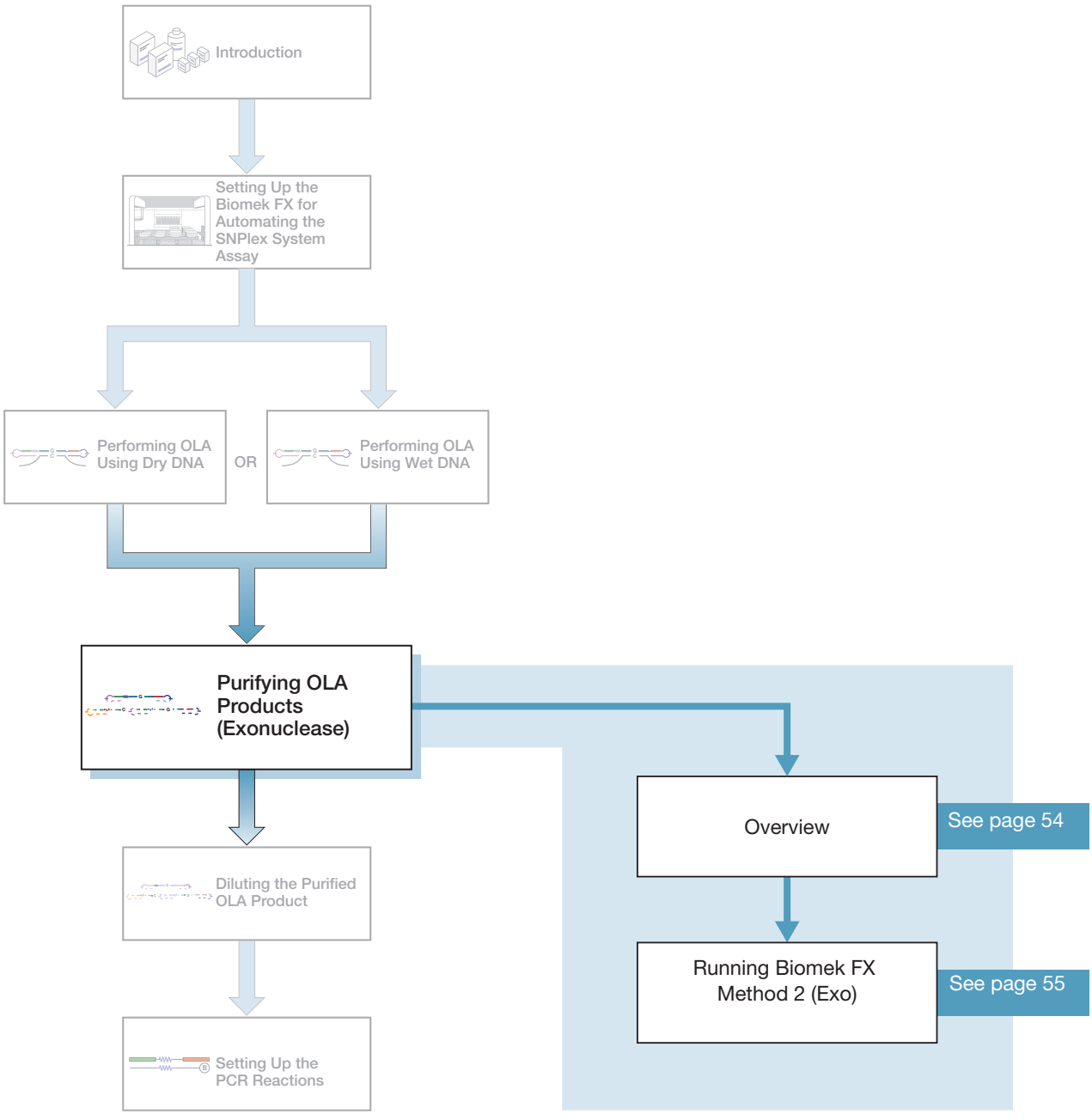
Step	Step Type	Temperature (°C)	Time
1	HOLD	48	30 min
2	HOLD	90	20 min
3	25 cycles	94	15 sec
		60	30 sec
		51, 3% ramp	30 sec
4	HOLD	99	10 min
5	HOLD	4	∞

Next Steps

At this point, the OLA reaction is complete. Proceed to Chapter 5, "Purifying OLA Products (Exonuclease)."

Notes

Purifying OLA Products (Exonuclease)


Introduction
Setting Up the Biomek FX for Automating the SNPlex System Assay
Performing OLA Using Dry DNA
OR
Performing OLA Using Wet DNA
Purifying OLA Products (Exonuclease)
Diluting the Purified OLA Product
Setting Up the PCR Reactions
Overview
See page 54
Running Biomek FX Method 2 (Exo)
See page 55

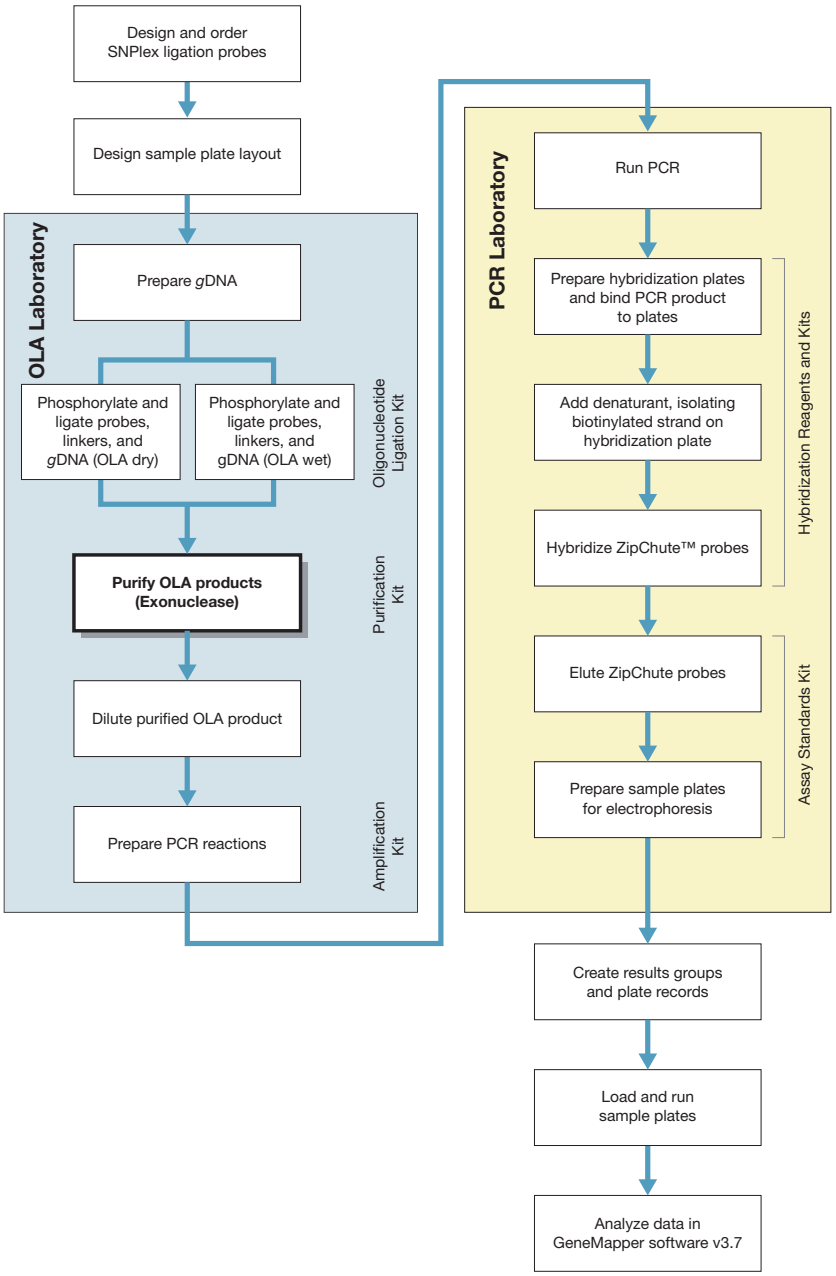
Notes

Overview

About This Chapter

This chapter provides information on automating the addition of the Exonuclease mix to the OLA reactions using the Biomek FX Instrument. The procedures in this chapter assume that you have completed the OLA preparation using either dry ("Performing OLA Using Dry gDNA" on page 19) or wet ("Performing OLA Using Wet gDNA" on page 35) gDNA.

Where You Are In the SNPlex System Assay Workflow


Design and order SNPlex ligation probes
Design sample plate layout
OLA Laboratory
Prepare gDNA
Phosphorylate and ligate probes, linkers, and gDNA (OLA dry)
Phosphorylate and ligate probes, linkers, and gDNA (OLA wet)
Oligonucleotide Ligation Kit
Purify OLA products (Exonuclease)
Purification Kit
Dilute purified OLA product
Prepare PCR reactions
Amplification Kit
PCR Laboratory
Run PCR
Prepare hybridization plates and bind PCR product to plates
Add denaturant, isolating biotinylated strand on hybridization plate
Hybridize ZipChute™ probes
Hybridization Reagents and Kits
Elute ZipChute probes
Prepare sample plates for electrophoresis
Assay Standards Kit
Create results groups and plate records
Load and run sample plates
Analyze data in GeneMapper software v3.7

Notes

Running Biomek FX Method 2 (Exo)

Assumptions

This method assumes that four 384-well OLA reaction plates (prepared in Chapter 3 or Chapter 4) have been prepared and thermalcycled.

About this Method

This method uses two deck layouts. The first deck layout adds Exonuclease mix to the OLA reaction plates OLA1 and OLA2. The second deck layout adds Exonuclease mix to OLA reaction plates OLA3 and OLA4. The well contents are mixed after each dispense of the Exonuclease mix.

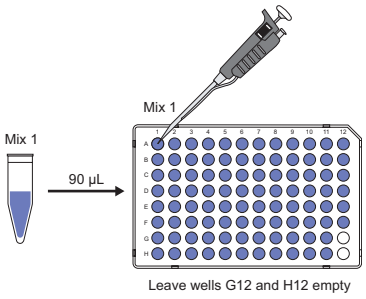
Preparing the Exonuclease Mix

1. Prepare an Exonuclease mix in a 15-ml centrifuge tube and mix thoroughly.

	Manual	Automated			
		Automation Losses		Automation Totals††	
Reagent	**Volume (µL) for One 384-well Plate†**	**Source Plate Dead Volume (µL) ‡**	**Transfer Loss Excess (µL)§**	**Total Volume (µL) for One 384-well Plate**	**Total Volume (µL) for Four 384-well Plates**
Nuclease Free Water	1579.2	789.6	453.6	2822.4	7560.0
SNPlex™Exonuclease Buffer	188.0	94.0	54.0	336.0	900.0
SNPlex™ Lamda Exonuclease	75.2	37.6	21.6	134.4	360.0
SNPlex™Exonuclease	37.6	18.8	10.8	67.2	180.0
Total	1880.0	940.0	540.0	3360.0	9000.0

† 376 reactions per plate × volume for one reaction.
‡ 94 tips in source plate × 10µL per tip.
§ Transfer losses for transferring bulk mixture into 96-well source plate.
†† Use the volumes in this column to prepare the reagents. Refer to the *SNPlex™ Genotyping System 48-plex User Guide* for per reaction volumes. Volumes given in this table include allowances for dead volume and excess volume for pipetting losses. For more information about calculating dead volumes, refer to the *SNPlex™ Genotyping System 48-plex General Automation Getting Started Guide* .

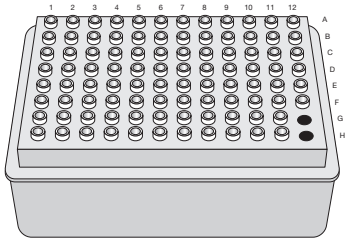
2. Add 90 µL of the Exonuclease mix into each well of a skirted 96-well plate, excluding G12 and H12, then centrifuge the plate briefly to collect the contents at the bottom of the wells.



Notes

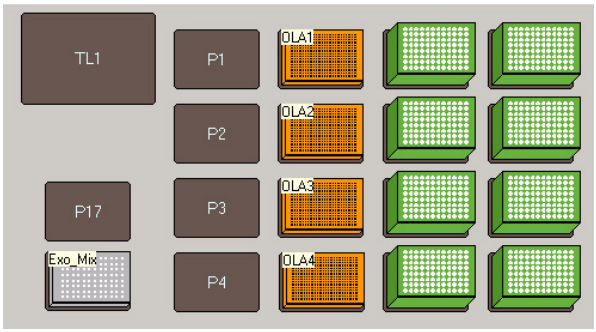
Running This Method

1. Remove the tips corresponding to well positions G12 and H12 from every tip box.



Remove tips from wells G12 and H12

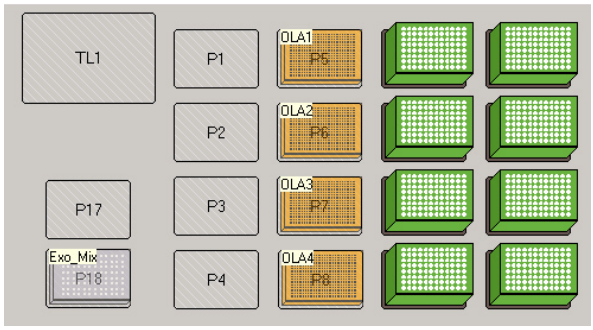
2. Place eight uncovered tip boxes and plates on the deck as shown in the following figure, placing an ACME adapter for the Thermal Exchange ALP underneath each OLA plate.

Plate	Position	Deck Layout 1
OLA1 to OLA4	P5 to P8	
Exo Mix	P18	
Uncovered tip box	P9 to P16	

3. Start the method using 2_SNPLEXv5_EXO.bmt.
4. When the method pauses, verify the deck layout and click **OK** to continue.
5. When the method pauses again, remove and discard the eight used tip boxes.

Notes

6. Remove the tips corresponding to well positions G12 and H12 from eight new uncovered tip boxes and place them on the deck as shown in the deck layout below.

Plate	Position	Deck Layout 1
OLA1 to OLA4	P5 to P8	
Exo_Mix	P18	
Uncovered tip box	P9 to P16	

7. Click **OK** to continue the method.

8. After the method completes, remove all eight used tip boxes and the Exo_Mix plate.

9. Remove, seal, and briefly centrifuge the OLA reaction plates (OLA1 to OLA4).

10. Proceed to "Thermal Cycling the OLA Reactions."

Thermal Cycling the OLA Reactions

Thermal cycle the OLA plates using the following conditions:

Step	Step Type	Temperature (°C)	Time
1	HOLD	37	90 min
2	HOLD	80	10 min
3	HOLD	4	∞

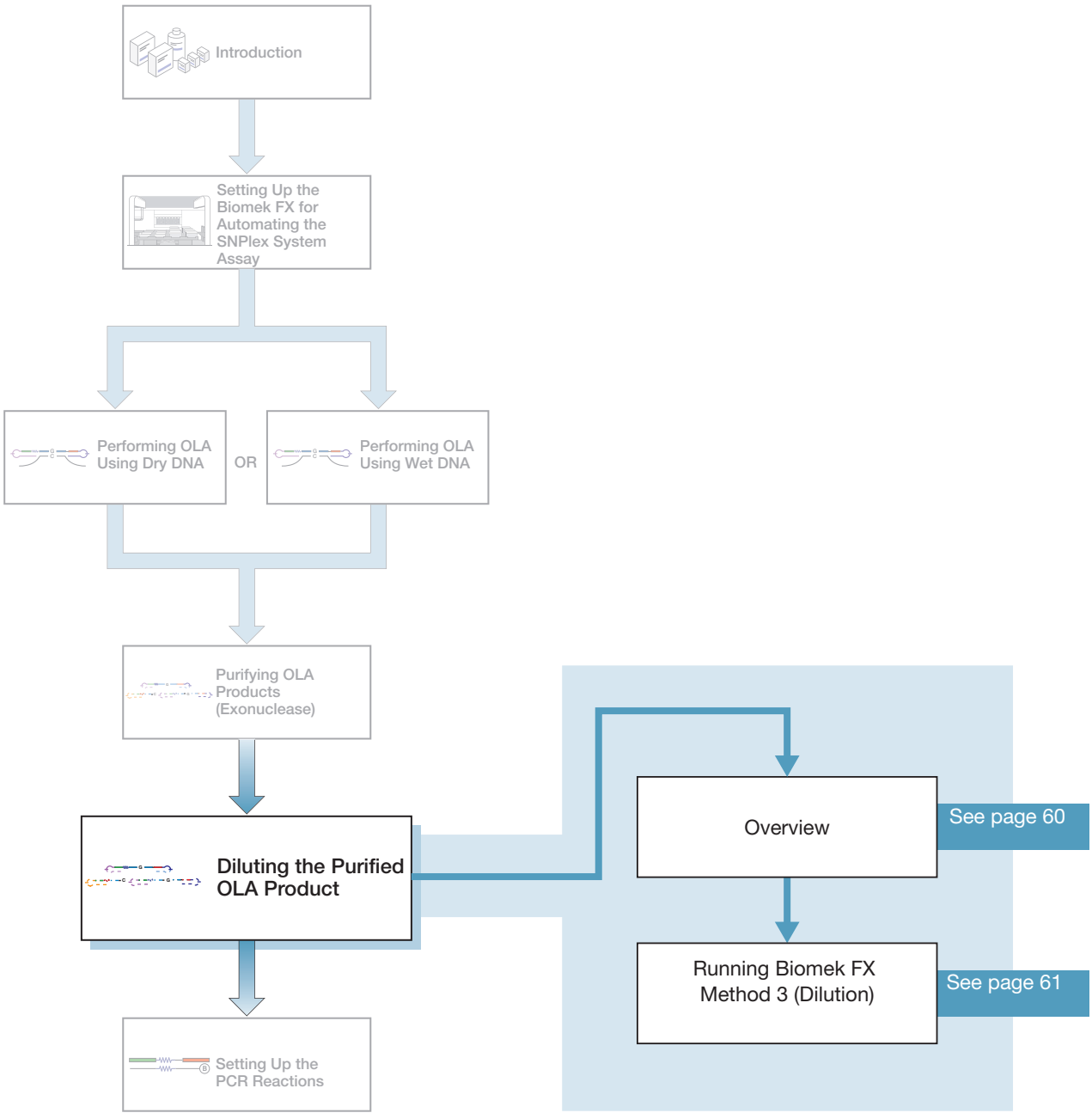
Next Steps

At this point, the OLA purification reaction is complete. Proceed to Chapter 6, "Diluting the Purified OLA Product."

Notes

Notes

Diluting the Purified OLA Product


Introduction
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OR
Performing OLA Using Wet DNA
Purifying OLA Products (Exonuclease)
Diluting the Purified OLA Product
Setting Up the PCR Reactions
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See page 60
Running Biomek FX Method 3 (Dilution)
See page 61

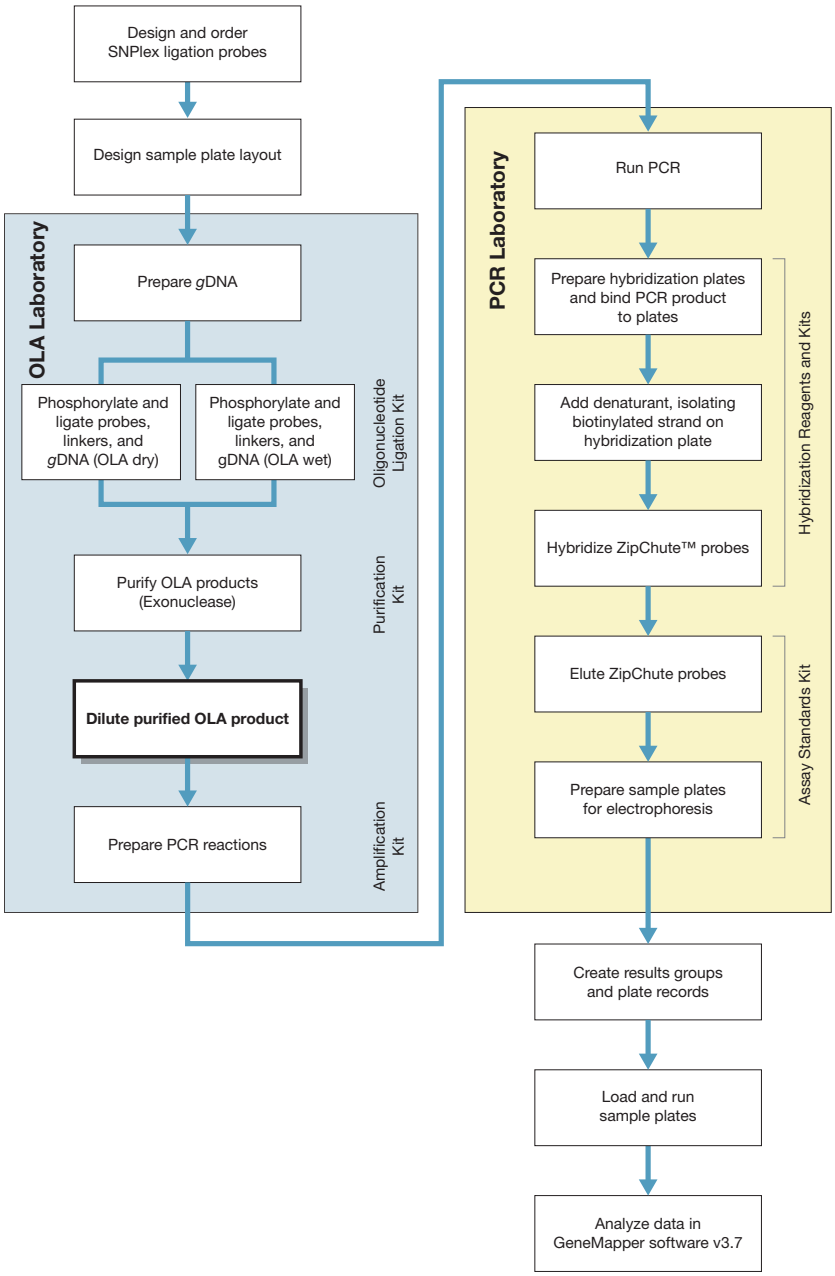
Notes

Overview

About this Chapter

This chapter provides information on how to dilute SNPlex OLA reaction plates, previously purified by exonuclease, using the Biomek FX liquid handling instrument to dispense nuclease free water.

Where You Are In the SNPlex System Assay Workflow


Design and order SNPlex ligation probes
Design sample plate layout
OLA Laboratory
Prepare gDNA
Phosphorylate and ligate probes, linkers, and gDNA (OLA dry)
Phosphorylate and ligate probes, linkers, and gDNA (OLA wet)
Oligonucleotide Ligation Kit
Purify OLA products (Exonuclease)
Purification Kit
Dilute purified OLA product
Prepare PCR reactions
Amplification Kit
PCR Laboratory
Run PCR
Prepare hybridization plates and bind PCR product to plates
Add denaturant, isolating biotinylated strand on hybridization plate
Hybridize ZipChute™ probes
Hybridization Reagents and Kits
Elute ZipChute probes
Prepare sample plates for electrophoresis
Assay Standards Kit
Create results groups and plate records
Load and run sample plates
Analyze data in GeneMapper software v3.7

Notes

Running Biomek FX Method 3 (Dilution)

About this Method

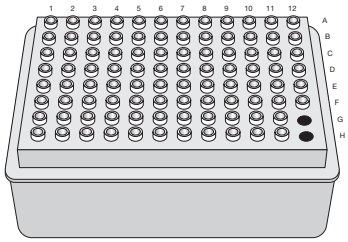
This method uses two deck layouts. The first deck layout adds water to OLA reaction plates OLA1 and OLA2. The second deck layout adds water to OLA reaction plates OLA3 and OLA4. The well contents are mixed each time after the water is dispensed.

Preparing the Reagent

Add 50 mL of nuclease-free water to an Axygen reservoir.

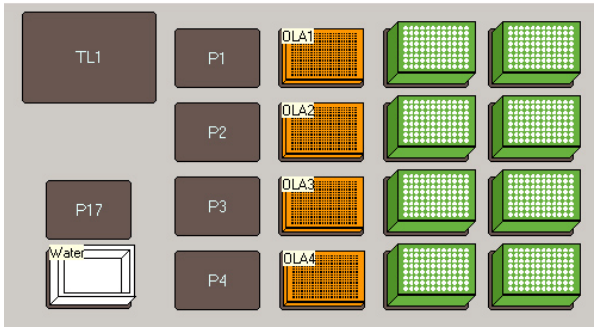
Running This Method

1. Remove the tips corresponding to well positions G12 and H12 from eight tip boxes.



Remove tips from wells G12 and H12

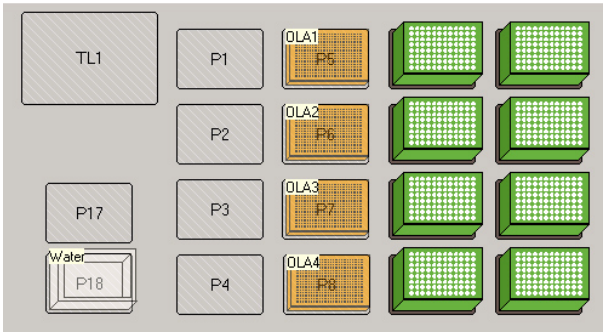
2. Place the eight uncovered tip boxes, the water reservoir, and plates on the deck as shown in the deck layout below.

Plate	Position	Deck Layout 1
OLA1 to OLA4	P5 to P8	
Water	P18	
Uncovered tip box	P9 to P16	

3. Start the method using 2_SNPLEXv5_EXO_DILUTION.bmt.
4. When the method pauses, click **OK** to continue.
5. When the method next pauses, remove and discard the eight used tip boxes.

Notes

6. Remove tips corresponding to well positions G12 and H12 from eight new uncovered tip boxes and place them and the plates on the deck as shown in the deck layout below.

Plate	Position	Deck Layout 2
OLA1 to OLA4	P5 to P8	
Water	P18	
Uncovered tip box	P9 to P16	

7. Click **OK** to continue the method.

8. After the method completes, remove all eight used tip boxes and the water reservoir.

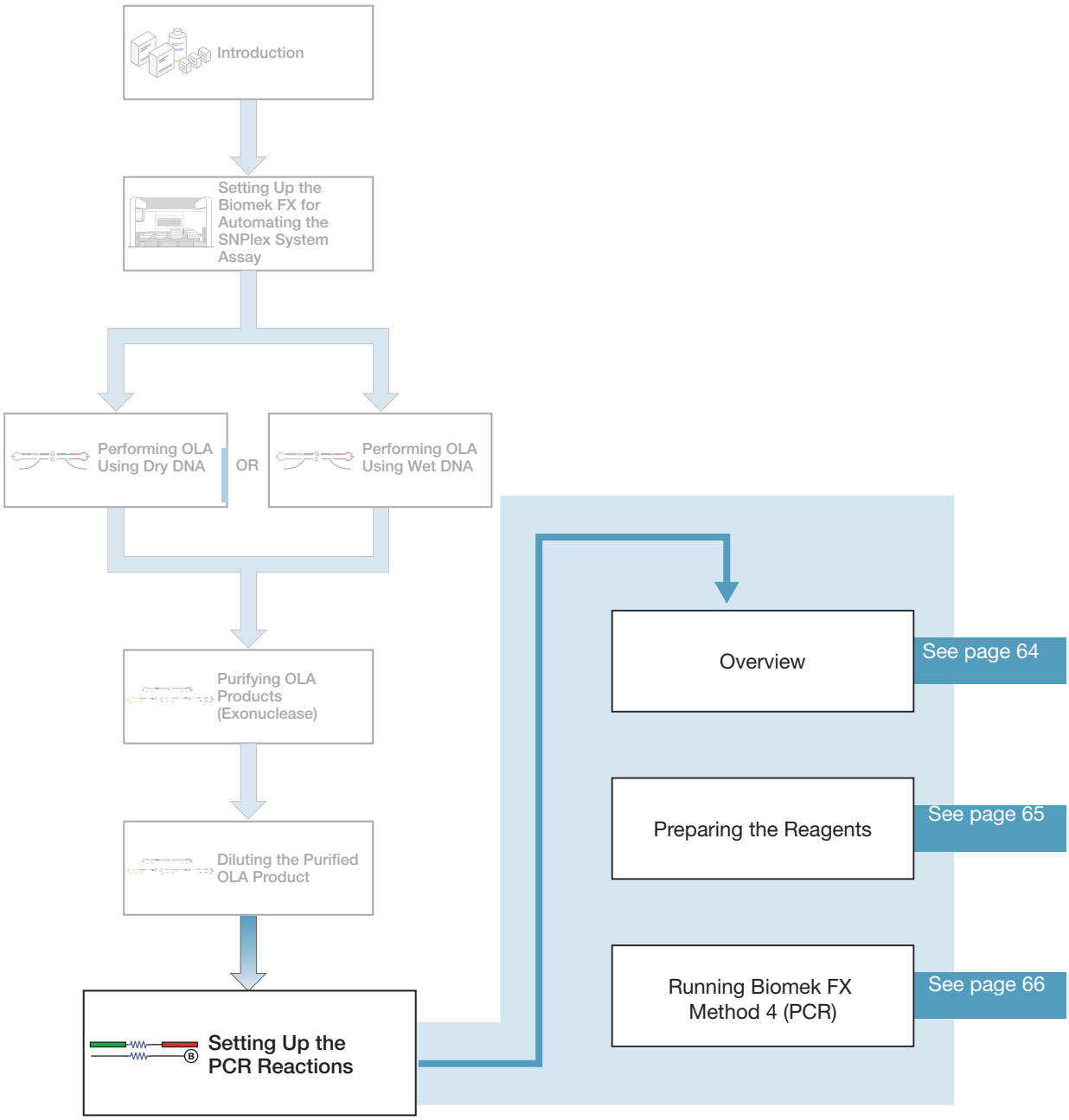
9. Remove, seal, and briefly centrifuge the OLA reaction plates (OLA1 to OLA4).

Next Steps

Store the plates if they will not be immediately used to prepare PCR reactions. Otherwise, return the plates to their positions on the deck to prepare PCR reactions. Proceed to Chapter 7, "Setting Up the PCR Reactions."

Notes

Setting Up the PCR Reactions


Introduction
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OR
Performing OLA Using Wet DNA
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Diluting the Purified OLA Product
Setting Up the PCR Reactions
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Preparing the Reagents
See page 65
Running Biomek FX Method 4 (PCR)
See page 66

Notes

Overview

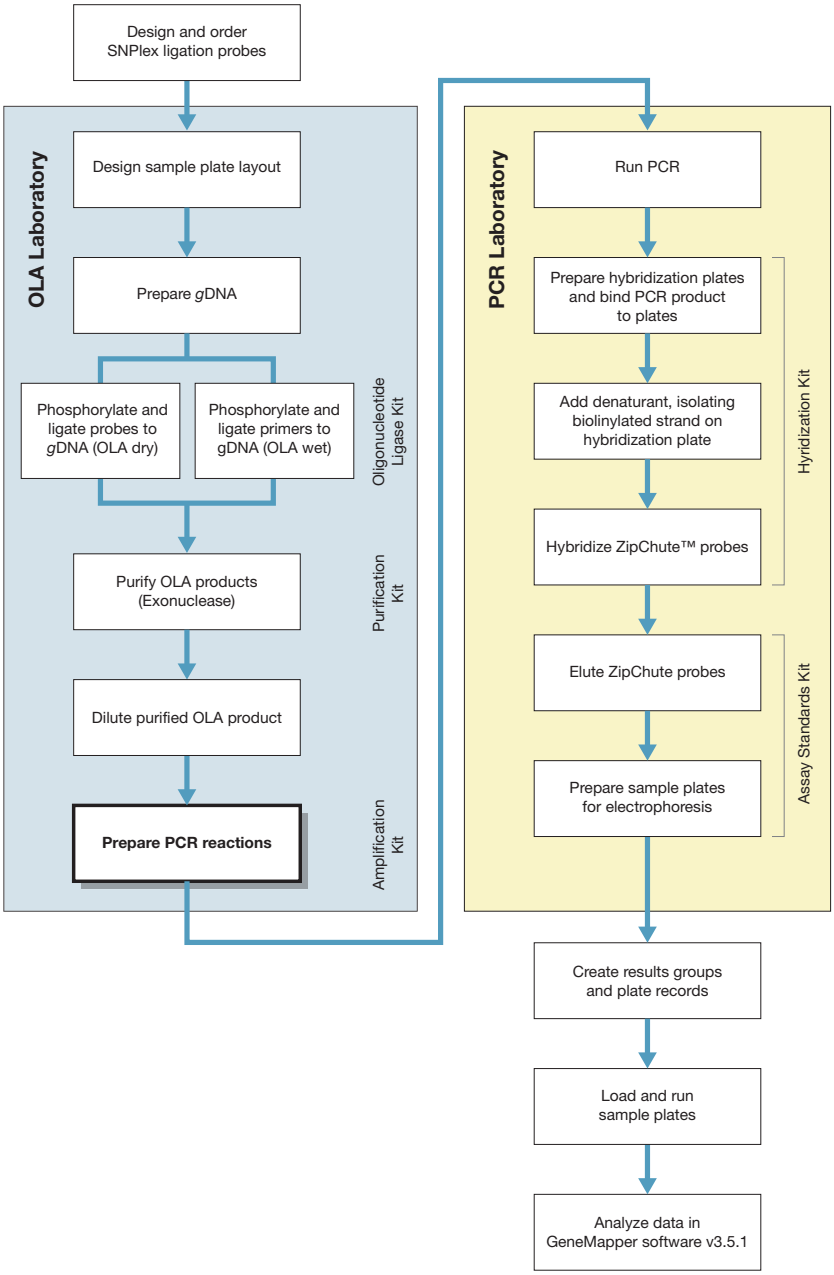
About this Chapter

This chapter provides information on how to prepare four SNPlex PCR reaction plates from four SNPlex OLA reaction plates using the Biomek FX liquid handling instrument.

Assumptions

This protocol assumes that four 384-well SNPlex OLA reaction plates are available and the contents previously exonuclease purified and diluted. One Biomek FX method is available for assembling the SNPlex system-based PCR reactions.

Where You Are In the SNPlex System Assay Workflow


Design and order SNPlex ligation probes
OLA Laboratory
Design sample plate layout
Prepare gDNA
Phosphorylate and ligate probes to gDNA (OLA dry)
Phosphorylate and ligate primers to gDNA (OLA wet)
Oligonucleotide Ligase Kit
Purify OLA products (Exonuclease)
Purification Kit
Dilute purified OLA product
Prepare PCR reactions
Amplification Kit
PCR Laboratory
Run PCR
Prepare hybridization plates and bind PCR product to plates
Add denaturant, isolating biolinylated strand on hybridization plate
Hybridize ZipChute™ probes
Hyridization Kit
Elute ZipChute probes
Prepare sample plates for electrophoresis
Assay Standards Kit
Create results groups and plate records
Load and run sample plates
Analyze data in GeneMapper software v3.5.1

Notes

Preparing the Reagents

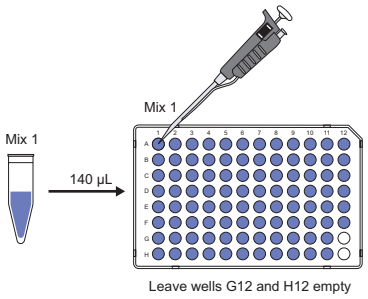
Preparing the PCR Mix

1. Thaw the SNPlex™ System Amplification Kit components at room temperature.
2. Label a skirted 96-deep well plate PCR Mix.
3. Label a 15-mL centrifuge tube.
4. Combine the following volumes of reagents in a 15-mL centrifuge tube and mix thoroughly.

	Manual	Automated			
		Automation Losses		Automation Totals	
Reagent	**Volume (µL) for One 384-well Plate†**	**Source Plate Dead Volume (µL) ‡**	**Transfer Loss Excess (µL)§**	**Total Volume (µL) for One 384-well Plate**	**Total Volume (µL) for Four 384-well Plates††**
Nuclease Free Water	909.9	287.2	198.1	1395.2	4125.0
SNPlex™ Amplification Master Mix	1880.0	593.4	409.3	2882.7	8522.7
SNPlex™ Amplification Primers	188.0	59.3	40.9	288.3	852.3
Total	2977.9	940.0	648.3	4566.2	13500.0

† 376 reactions per plate × volume for one reaction.
‡ 94 tips in source plate × 10µL per tip.
§ Transfer losses for transferring bulk mixture into 96-well source plate.
†† Use the volumes in this column to prepare the reagents. Refer to the *SNPlex™ Genotyping System 48-plex User Guide* for per reaction volumes. Volumes given in this table include allowances for dead volume and excess volume for pipetting losses. For more information about calculating dead volumes, refer to the *SNPlex™ Genotyping System 48-plex General Automation Getting Started Guide*.

5. Add 140 µL of the PCR master Mix into each well of a skirted 96-well PCR plate, excluding wells G12 and H12, then centrifuge the plate briefly to collect the contents at the bottom of the wells.



6. Label four 384-well ABI PRISM® clear optical reaction plates PCR1 to PCR4.

Notes

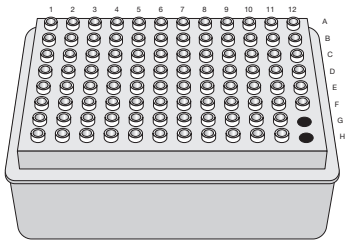
Running Biomek FX Method 4 (PCR)

About this Method

This method uses two deck layouts. The first deck layout adds PCR mix to all PCR reaction plates PCR1 to PCR4 and transfers aliquots from OLA1 and OLA2 to PCR1 and PCR 2, respectively. The second deck layout transfers aliquots from OLA3 and OLA4 to PCR3 and PCR4, respectively.

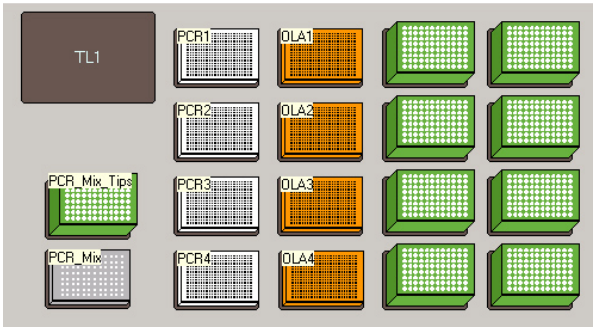
Running the Method

1. Remove the tips corresponding to well positions G12 and H12 from nine tip boxes.



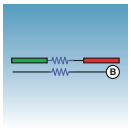
Remove tips from wells G12 and H12

2. Place an ACME adapter underneath each PCR plate, then place the nine uncovered tip boxes and the plates on the deck as shown in the deck layout below.

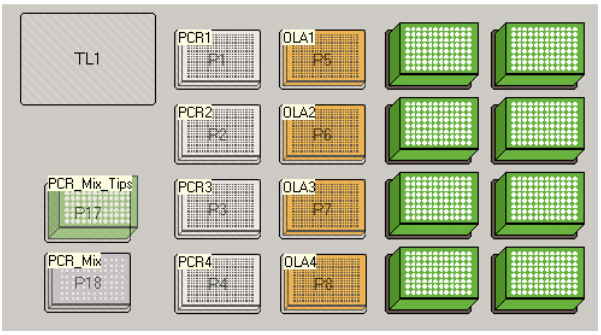
Plate	Position	Deck Layout 1
PCR1 to PCR4	P1 to P4	
OLA1 to OLA4	P5 to P8	
PCR Mix	P18	
Uncovered tip box	P17, P9 to P16	

3. Start the method using 2_SNPLEXv5_PCR.bmt.
4. When the method pauses, click **OK** to continue.
5. When the method next pauses, remove and discard the eight used tip boxes.

Notes



6. Remove the tips corresponding to well positions G12 and H12 from eight new uncovered tip boxes and place them on the deck as shown in the deck layout below.

Plate	Position	Deck Layout 2
PCR1 to PCR4	P1 to P4	
OLA1 to OLA4	P5 to P8	
PCR Mix	P18	
Uncovered tip box	P17, P9 to P16	

7. Click **OK** to continue the method.

8. After the method completes, remove the used tip boxes and discard the PCR Mix plate.

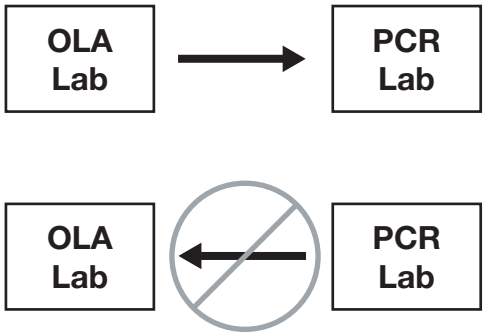
9. Remove, seal, and store the OLA reaction plates.

10. Remove, seal, and briefly centrifuge the PCR plates (PCR1 to PCR4).

Next Steps

At this point, move the PCR plates to the PCR lab to complete thermal cycling and the rest of the steps in the SNPlex System assay workflow.

IMPORTANT! Never move equipment, containers, or other items from the PCR Laboratory or data collection area into the OLA Laboratory.



Notes

Notes

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Headquarters
850 Lincoln Centre Drive
Foster City, CA 94404 USA
Phone: +1 650.638.5800
Toll Free (In North America): +1 800.345.5224
Fax: +1 650.638.5884

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